1974

A Cytological Analysis Of Sibling Species Of Simulium Damnosum (diptera: Simuliidae) In West Africa

Charles Gbilekaa Vajime

A CYTOLOGICAL ANALYSIS OF SIBLING SPECIES OF *SIMULIUM DAMNOSUM* (DIPTERA: SIMULIIDAE) IN WEST AFRICA

by
Charles Gbilekaa Vajime

Department of Zoology

submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Graduate Studies
The University of Western Ontario
London, Canada

October, 1973

TABLE OF CONTENTS

A B S T R A C T

Cytological analysis of the banding pattern of polytene, salivary gland chromosomes was conducted on West African members of the Simulium (Edwardsellum) damnosum complex, vectors of onchocerciasis. S. damnosum shows three polytene chromosomes in each salivary gland nucleus.

Seven sibling species, five new and two previously known were identified on the basis of one or more conventional criteria: distinctive interspecific inversions, discrete sex chromosomes and restricted inversion polymorphism. "Nyamagasani" of the East African "Sanje" subgroup was used as the standard for the West African "Nile" subgroup.

On the grounds of shared rearrangements, these species comprise three categories: "Bille-Yah", "Bandama-Soubre" and "Nile-Sirba-Dieguera".

"Bille-Yah"

"Bille" and "Yah" share the fixed inversions IS-1 and IL-3. "Bille" has sex chromosomes designated as $X_0Y_0Y_1$, one kind of Y chromosome (Y_0) being indistinguishable from the X(X_0), the other Y being marked by a modification of the centromere region of chromosome I. In "Yah" the sex chromosomes are IIL standard(Y_0) and IIL-18 (X_1 Y_1).

The floating inversions IS-II, IL-15, IIIS-5 and IIIL-16 are restricted to "Bille" while IIL-40 is confined to "Yah". Only IIS-6 is shared.

"Bille" breeds in rivers, "Yah" in creeks. "Bille" is known from both forest and Savannah regions. "Yah", however, was not found outside the forest region. "Bille" is considered a vector of onchocerciasis. The role of "Yah" as a vector is not clear.

"Bandama-Soubre"

"Soubre" is defined by IIL-6, "Bandama" by IIL-6.7. A few individuals heterozygous for this inversion persist in populations of both species.

The floating inversions IIIL-24, IIIL-25 and IIIL-26 are restricted to "Soubre" while IS-20, IS-21, IL-18, IIIL-5, IIIL-23 and IIIL-30 are restricted to "Bandama".

The inversions shared are the fixed IL-6, IIL-4 and IIIL-4, the virtually fixed IIIL-2.17 and the floating IIIL-2/IIIL-2.17, IL-1, IS-5, IIS-7 and IIL-4.39.

"Soubre" is known from both forest and Savannah regions. "Bandama" was not found beyond the forest zone. Both species breed in large rivers. "Soubre" is considered a vector of onchocerciasis. The vectoral role of "Bandama" is questionable.

"Nile-Sirba"

"Nile" and "Sirba" share the fixed inversions IS-1 and IL-3 with the other five sibljngs.

This pair has the fixed inversion IIIL-2 common with "Bandama" and "Soubre".

"Nile" and "Sirba" are characterized by a complex rearrangement in the IIL arm designated as IIL-C and by the fixation of IL-1.

"Nile" is defined by XY sex chromosomes based on an inversion in chromosome II; i.e. IIL-C.8, and "Sirba" by sex chromosomes based on an inversion in chromosome I, i.e. IS-3. Two "sub-siblings" of "Sirba" are recognized; in one the X chromosome (X_0) is marked by the standard arrangement, in the other the X chromosome (X_1) is marked by the inversion IS-3.

The floating inversions IS-16 and IS-12 are confined to "Nile" while IIIL-22 and IIIL-27 are exclusive to "Sirba".

Both have in common IS-2, IS-2.18, IL-2, IL-13, IIL-20, IIL-35 and IIIL-7.

The range of these widely distributed species extends from the forest region where "Nile" predominates to the Sudan Savannah which is almost exclusively a "Sirba" zone. Both species are considered as transmitters of onchocerciasis.

"Dieguera"

"Dieguera" is distinguished by the fixation of IS-1 IS-2, IL-3, IIL-C.8 and IIIL-2. There is no information on the biology of this species.

In brief, cytological aspects of all species are presented in idiograms. The phylogenetic interrelationships of the species are summarized in a chart.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Prof. R.W. Dunbar and Prof. K.H. Rothfels (Botany Department of University of Toronto) for their generous help and supervision of this investigation. I am grateful to Prof. J. George for his valuable advice and keen interest.

Thanks are due to Prof. K. Misra and Prof. D.B.W. Reid (Biometrics Department, University of Toronto) for their useful suggestions on statistical analysis.

The author appreciates the assistance of all those who supplied larval specimens - especially Dr. R. Garms of Bernhard-Nocht Institute Hamburg, Germany; Dr. B. Philippon and Mon. Sechan of OCCGE Ivory Coast; Mr. F. Walshs of W.H.O. Bolgatanga, Ghana; Dr. B.O.L. Duke, Dr. Disney and Mr. B. Thompson of Helminthiasis Research Institute Kumba, Cameroon.

I am obliged to Mr. D. Bedo for his help with photography and for proof-reading the text. The full co-operation of the staff of Prof. Rothfel's laboratory is appreciated.

Financial support is acknowledged in the form of grants in aid of research to Prof. Dunbar from The National Research Council of Canada and the World Health Organization; financial aid from the Zoology Department, University of Western Ontario is very much appreciated.

LIST OF PHOTOGRAPHIC PLATES -DESCRIPTION

LIST OF TABLES

LIST OF MAPS

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

Simulium - Onchocerca Background

Black flies (Diptera, Simuliidae) are of world-wide distribution and black fly females are among the most oppressive blood-sucking insects known which torment man as well as animals. In North America and Europe, where large swarms molest cattle, the resulting deaths from severe toxaemia and loss of blood sometimes add up to many thousands of dollars a year as stated by Chandler and Read (1967). In parts of Africa, Mexico and Central America, Crisp (1956), Le Beere (1966) and Chandler (1967) have reported that some black fly species are intermediate hosts and vectors of filarial worms which cause onchocerciasis, a debilitating, blinding disease in man.

The genus Simulium contains all known species that are onchocercal hosts as reported by Chandler et al (1967): S. ochraceum, S. metallicum and S. callidum in Mexico and Central America, S. neavei and S. damnosum in Africa. The first record of species of this affinity in Africa was from the Nile valley of Uganda by Theobald (1903). From Sierra Leone on the western coast, Blacklock (1926) showed that the dreaded, filarial parasite of man, Onchocerca volvulus was transmitted by S. damnosum, which has since been recognized by Crosskey (1969) as belonging to Simulium (Edwardsellum) with three known species; two of these S. machadoi Luna

de Carvalho and S. vilhenai Luna de Carvalho have been reported only from Angola in Central Africa; the third S. damnosum, is known from Yemen as published by Merighi and Parrinello (1969) and from numerous localities in northern, central, eastern and western Africa.

In West Africa Noamesi (1964), Davies (1963, 1968, 1969), Duke, Lewis and Moore (1966), Lewis and Duke (1966), Garms and Post (1966), Garms and Weyer (1968), Knuttgen (1964), Le Berre (1966) and Germain, Grenier and Mouchet (1968) have presented detailed information on the occurrence of the fly. The general distribution of S. damnosum outlined by these workers does not completely coincide with that of onchocerciasis because in some areas S. damnosum does not appear to bite man as suggested by Garms (1973) and Garms and Voelker (1969). Endemic areas for onchocerciasis include a huge belt across Africa that extends from 15 degrees North to 15 degrees South.

In recent years, the increasing realization of the importance of the disease has resulted in intensive Onchocerca-Simulium studies in the hope of facilitating control programs. In West Africa, epidemiological differences are known to exist between the humid coastal forest and the almost arid northerly Savannah regions as reported by Le Berre, Balay, Brengues and Coz (1964). Duke et al (1966) after studying the development of Onchocerca volvulus from the forest in S. damnosum from the Savannah and vice versa, consider that "there are in West Africa two strains of O. volvulus and two main physiological forms of S. damnosum, with some minor variations..." Two of the co-authors Lewis and Duke (1966) have reported three classes,

A, B, C, of female S. damnosum on the basis of colour; the Savannah zone has a preponderance of class C, pale flies, as opposed to class A, dark flies, in the Southern forest region. In addition they state that, "the average wing length is greatest in certain Southern areas". Disney (1969) has utilized details of abdominal scales to propose two larval forms for the forest region of West Cameroon. In Liberia, Garms (personal communication 1971) has observed that larval populations in small rivers have shorter dorsal, abdominal conical bumps than those in large rivers.

The bio-ecology of S. damnosum is as intriguing as its morphology. Le Berre et al (1964) have noted how life expectancy of females increases from the coastal forest to the Savannah while their dispersal ranges decrease in the same direction. Some are anthropophilic, others zoophilic as reported by Garms et al (1969), Garms (1973) and by Disney and Boreham (1969).

Such complex array of information has strengthened the suspicions of those studying black flies that S. damnosum is probably not a single species. But definitive taxonomic investigations employing conventional methods have so far been hampered by the lack of observable, specific features among populations of the fly. The present study is an attempt to characterize S. damnosum populations cytotaxonomically. It is hoped that this approach will throw some light on the diversity of black flies in West Africa.

Cytotaxonomic Background

Cytotaxonomic studies of the banding patterns of larval salivary gland chromosomes have been very useful in the study of closely related species groups or sibling species complexes in the Simuliidae and other dipteran insects.

Among Simuliids information on cytological delimitation of species is presented by Basrur (1959) in Prosimulium, Dunbar (1959) in Eusimulium aureum , Madahar (1969) in Cnephia (Stegopterna) and Landau (1962) in Simulium tuberosum.

This cytological approach depends primarily on the rearrangements of a discernable banding sequence which is as close to the primary genetic structure as one can observe with the light microscope. Since giant, polytene salivary gland chromosomes are at the most indirectly exposed to the external environment, the effects of evolutionary convergence are minimized and similarities may be considered to indicate real phyletic relationships.

Simulium damnosum has received previous cytotaxonomic attention through Dunbar's (1966, 1969) work in East Africa, where he has reported nine cytological segregates in two subgroups : "Sanje" and "Nile". McCrae's (1969) correlation of these segregates with both known areas of onchocerciasis foci and morphological larval features have shown that "Dunbar's cytotaxonomic studies illuminated long-recognized but confusing problems of anthropophilism and non-anthropophilism..."

It is therefore of more than academic interest to find out if a similar situation exists in West Africa; that is, the existence of sibling species definable cytologically and differing in aspects of their biology and capacity to act as vectors of Onchocerca volvulus. Dunbar's preliminary studies (1969) suggested the existence of two cytological segregates, "Nile and Bandama". The present study though limited in scope suggests that there are no less than seven taxa subsumed under S. damnosum in West Africa.

MATERIALS AND METHODS

The material on which this investigation is based came from three main geographic regions of West Africa: the hot humid coastal forest belt, the two central Guinea Savannah zones and the almost arid, northernly Sudan Savannah region. These ecological zones are not sharply demarcated. Transitional areas as well as areas of different vegetation within a major zone such as a forested valley along a river in a Savannah region exist. The "Dahomey Gap" for instance is an intrusion of Savannah into the forest.

Collection Sites

Details of collection data are shown in table 10. A summary of locations featuring different ecological areas is presented below:

(1) Sudan Savannah :

Sudan Mali.

(2) Northern Guinea Savannah:

Northern Ghana

(3) Southern Guinea Savannah

Southern Ghana

Central Nigeria

(4) "Dahomey Gap"

Dahomey

(5) Bandama Valley (Forest)

Ivory Coast

(6) Hilly forest country

Ivory Coast..Mt. Nimba

Liberia..Bong Hill

Cameroon.. Kumba area

(7) Dense forest country

Southern Ivory Coast

Liberia

For the description and taxonomy of all stages of S. damnosum consult Crosskey (1969). In brief, larvae may be recognized by paired conical bumps in the first five abdominal segments. The pupa is characterized by a shoe-shaped cocoon and eleven respiratory filaments organized into two lateral, three outer and six inner filaments. The adult is characterized by a hairy basal section of the radius, a bare pleural membrane and katepisternum and by a greatly dilated tarsus.

S. damnosum larvae are usually found attached to rocks or vegetation in fast running water. For cytological analysis, 5th instar larvae with white respiratory histoblasts are preferable though larvae of

other stages that show discernable, polytene chromosomes may be used. Larval samples with which this investigation was begun were sent to Canada by collectors from West Africa. These larvae produced consistently unsatisfactory chromosome preparations.

As a result of observations on the effect of temperatures around 0°c on slide preparations, the "ice collecting method" since published by Dunbar (1972) was applied to African larvae during a collection trip in 1971.

This technique involved the use of a cooling device, ice or a cooler, to immobilize larvae as soon as they were collected. Ice was taken to collecting sites in a thermos flask, large enough to contain both ice blocks and supporting accessories for the larvae. Larvae were collected in small-sized petri dishes lined with soaked cotton wool or in plastic bags sufficiently perforated to facilitate air circulation. The whole assemblage was placed on ice blocks in the flask which was loosely corked to enhance air circulation. Larvae were kept alive in this condition for at least twenty-four hours and then fixed.

Where long field trips were undertaken, and larval mortality in transit was anticipated, the material was fixed in the field. This required placing the larvae directly on ice blocks for about thirty minutes before fixation.

Where a cooler was used as the immobilizing device, larvae were collected in beakers containing some water from the same breeding site.

Pieces of vegetation in the beakers served as useful support for the larvae. The whole assemblage was placed in the cooler. In the laboratory the material in the beakers was kept overnight in a refrigerator and the larvae fixed the following day.

Immobilized tropical larvae assume an S. shape, and usually show no signs of movement at all. Live larvae always wriggle when in contact with a drop of the fixative. This reaction was used to distinguish dead larvae from the desired live ones.

Dissection and Fixation

Salivary glands are large, transparent, paired structures that extend posteriorly to about two-thirds of the body length and then curve back as shown by Puri (1925).

To expose the glands, a larva was placed on a slide, excess water blotted from the specimen and the animal quickly ruptured laterally with sharp pins or forceps. Usually the large glands were seen popping out.

Each dissected specimen was immediately placed in freshly prepared Carnoy's fixative (one part acetic acid; three parts absolute ethanol), the amount of fixed material not exceeding five to ten percent by volume. Fixed larvae were stored in the refrigerator.

Salivary Gland Chromosome Preparations

Fixed larvae were processed according to the standard procedures of Rothfels and Dunbar (1953) and Dunbar (1972). After rinsing in distilled water for about ten minutes to remove excess Carnoy's fixative, the specimens were hydrolysed in 1.0 N HCl at 60°c for seven minutes.

Specimens were stained in a tightly stoppered vial of Feulgen stain until the salivary gland nuclei were observed to be bright red. The usual time for the completion of this process varies from fifteen minutes to five hours. Specimens that failed to stain within this time were left in stain overnight in a refrigerator.

Temporary Mounting

After rinsing the specimens in three changes of tap water of three minutes each to remove excess stain and to harden the chromosomes, larvae were placed on a slide, one at a time, in a drop of 50% acetic acid. Using a dissecting microscope and fine needles, the glands and gonads identified as dark-staining pear-shaped testis or long tapering ovaries situated dorsally near the mid gut were collected together, the carcass and all excess material removed, a cover slip applied and the glands along with the gonads gently squashed in a drop of 50% acetic acid.

At this point the preparation was examined. If the degree of spread of the chromosomes was not satisfactory, the slide was squashed

again and if the chromosomes were inadequately stained the stain was intensified by applying a drop of orcein in 50% acetic acid under the cover slip.

Permanent Mounting : The Dry Ice Technique

The temporary slides were placed with the cover slip down on dry ice for about five minutes. The cover slip was gently removed using a razor blade and both the slide and the cover slip were immediately dipped in a jar of 95% ethanol to dissolve the crystals of ice and acetic acid. After a rinse in absolute ethanol, the slide and the cover slip were reunited with a drop of euparal. Excess euparal was blotted away and the preparation left to dry for about one week.

Epon Epoxy Resin Technique

Where a large number of slides were to be made permanent, epon was used as the mounting medium. In addition, it proved advantageous in that there was no fading of tissue. Details of this technique have been reported by Dunbar (1972). In brief the resin consists of a glycerol-based epoxy resin, "Epon 812" (Shell Chemical Co. trademark). It is set with two liquid anhydrides, DDSA (dodecenyl succinic anhydride) and NMA (nadic methyl anhydride). An accelerator, DMP (2, 4, 6 -tri(dimethyl-aminomethyl) phenol) is used.

Two stock mixtures were prepared:

1.	Epon 41.3 cc	2.	Epon 39.5 cc
	DDSA 66.7 cc		NMA 32.5 cc

The mounting medium was prepared from 6 parts of mixture 1, 4 of mixture 2 and 7 drops of DMP. After thorough mixing, bubbles were removed under vacuum and the medium stored in plastic syringes in a freezer. For use, the syringes were allowed to warm up to room temperature. To remove tiny droplets introduced by this medium, the preparations were subjected to a vacuum for about ten minutes; excess epon was blotted away and the slides heated in an oven at 100°C for about thirty minutes after which the preparations were permanently ready.

Epon sets so hard that it can permanently bind slides together. Therefore before slides were piled in the oven, care was taken to ensure that no epon was on any parts in contact.

Microphotography

Microphotographs were taken with 35 mm camera fitted on a standard Universal Microscope or a Reichert Microscope. Kodak Panatomic was used for giant chromosomes, Kodak High Contrast Copy for meiotic chromosomes.

Prints were made on Kodabromide F1 to F5 to give the desired contrast. Photographic chromosome maps were prepared. Where necessary, composites of photographs that showed particular sections of the chromosomes most clearly were used.

Chromosomal Comparisons

In order to place East and West African S. damnosum siblings in a common phylogeny, a central arrangement, "Nyamagasani", a member of the East African complex was arbitrarily chosen as the main standard. In West Africa, "Bille" which is phylogenetically closest to "Nyamagasani" is considered the base.

Idiograms

Salivary gland chromosome idiograms (plate 2) are based on percent total complement length (TCL). Low power microphotographs of six evenly stretched nuclei, each from a different slide were prepared and chromosome arms measured using a map measurer. The average length of each arm was expressed as a percentage of the TCL.

Nomenclature

The three polytene chromosomes of S. damnosum are numbered I, II, and III in decreasing order of length. The letters S and L refer to the short and long arms.

To describe chromosome regions and facilitate detailed analysis, standard chromosomes were divided into one hundred sections of approximately even sizes, starting with section one at the IS end through to section one hundred at the IIIL end. Where necessary, each section was further divided into subsection, A, B, and C. Within a subsection bands were assigned consecutive numbers. Thus the first band in the first

subsection of section one would be IA1, the second IA2 etc. Limits of sections and subsections are shown in Plate 3, Fig. 12, Plate 4, Fig. 19, and Plate 5, Fig. 26.

Inversions in each arm were identified by different numbers in order of their discovery. Interspecific or fixed inversions such as IS-1 and IL-3 are underlined. Intraspecific or floating inversions are not underlined. Rearrangements which differ from the standard sequence by two or more steps are indicated by a numbering system which takes into account the order in which rearrangements occurred Thus in IS-2.18., "2" signifies the first step involved in the rearrangement, while "18" indicates a subsequent overlapping or included inversion. Independent inversions of the same arm are indicated by a + as in IL-1+3.

CHAPTER 2

DESCRIPTION OF CHROMOSOMES

General Description of Complement : Landmarks

Meiotic chromosomes of male gonads presented in Plate 1, Fig. 1 show that S. damnosum is chiasmate. In each nucleus there are three bivalents of unequal size that are identified as chromosomes I, II and III in decreasing order of size. The primary constrictions or centromeres approximate those of the three giant polytene chromosomes in the salivary gland nuclei shown in Plate 1, Fig.2.

The average arm ratios of the giant, polytene chromosomes are1:1.1,1:1.5 and 1:1.8 for chromosomes I, II and III respectively as reported by Dunbar (1966) in S. damnosum.

Each salivary gland chromosome is characterized by key landmarks. The relative positions of these markers associated with each chromosome are shown in the idiograms, in Plate 2 and in the photographic Plate 3 to 7. It can be seen that each chromosome exhibits tight pairing. The outstanding "micro-morphological" features of the chromosomes include: the expanded region in chromosome I, the nucleolus and "capsule" in the short arm of the same chromosome, the "double bubble" and "Balbiani Ring" in IIS, the "Para Balbiani" in IIL, the "blister" in IIIS and the centromeres.

The banding pattern itself is a unique feature.

Description of Chromosomal Landmarks

Excepting the centromeres usually expressed as an aggregation of dark-staining bands, the chromosomal markers listed above are recognized as "puffs" interpreted as structural modifications of bands at chromosome regions associated with differential gene activity as reported by Beermann and Clever (1964) in Chironomus tentans and Berendes (1968) in Drosophila hydei.

The value of these landmarks as diagnostics lies in the relative ease with which they can be spotted in interspecific rearrangements but especially in their attesting to the genetic affinity among and between species of dipteran genera in which polytene chromosomes are known.

Centromere

C_I, C_{II} and C_{III} were identified by their homologies with "Nyamagasani" and also by the finding of centric, ectopic pairing and centric fusion in "Yah" (Plate I, Figs. 3,4), a member of S. damnosum species group. Centric fusion is known from other simuliids as reported by Rothfels (1956) in Prosimulium multidentatum and Dunbar (1969) in "Sanje", Nkuski" and "Ketaketa" of the "Sanje" subgroup.

Expanded Region

This landmark is located in section 21 of chromosomeI. It is "compact", approximately three times larger in diameter than the

rest of the chromosome and is generally the darkest staining region of the entire complement. This globular region appears to consist of a net work of dark, granular bodies organized loosely into transverse heavy bands. The most organized of these bands are assumed to constitute the centromere (Plate I, Figs.2,4). Between interband regions can be observed in light stained preparations, fibrous longitudinal strands. A study of the expanded region of several Simulium species has led Rothfels and Dunbar (1953) to interpret "expanded regions as being essentially like the rest of the chromosome except that the structure is locally modified by excessive size chromomeres....." and that the region is probably heterochromatic.

In S. damnosum interspecific group homology of this region is shown in Plates 2 and 3. Zimring (1953) has reported similar observations in several North American simuliid species.

Nucleolus

This marker is located in section 19 of the short arm of chromosome I. In Feulgen stained material, the nucleolar area exhibited a diffuse, clumpy appearance. The heavy "spiky" group of bands in 19A probably represents the attachment point of the nucleolus because where the nucleolus is expressed heterozygously, this tight group of bands ruptures as shown in Plate 3, Fig. 13.

In the West African "Nile" subgroup therefore, the attachment of the nucleolus appears to be interspecifically constant and

all species are mononucleolar. In the East African "Sanje" subgroup, Dunbar (1966) reported a secondary nucleolus in the IIIL arm of "Sebwe B".

"Capsule"

The "capsule" is located in 12A of the short arm of chromosome I. Its interspecific homology is presented in Plate 3, Figs. 12 to 18. The marker consists of two large, adjacent blocks of deeply staining bands with saw-toothed opposed edges as shown in Plate 3, Figs. 14 and 16. Details of its morphology are variable but the general configuration has always been observed conspicuously in S. damnosum. Because of such prominence, the "capsule" is a good marker for the IS arm as well as the rearrangement, IS-2, which shifts the position of this landmark (Plate 3, Figs. 14 and 18).

"Double bubble"

The "double bubble" was first reported by Landau (1953) in Simulium tuberosum. This marker has been found homologous with that in the S. damnosum species group. It is located in 51C and 52A of the short arm of chromosome II (Plate 5, Figs. 26, 27, 30).

The "double bubble" is usually characterized by a"puff" on which a configuration resembling two bubbles is imposed by two central light bands. Where "puffing" is lacking as shown in Plate 6, Fig.32, the region is characterized mainly by three light double bands. Where "puffing" occurs, no organized bands were observed, instead a cottony, fibrous mass was noticed as presented in Plate 6, Fig.31.

"Ring of Balbiani"

This is the largest "puff" in the entire complement. It was discovered by Balbiani in 1881 and has been extensively studied by Beermann and Clever in Chironomus tentans. Similarly the largest "puff" in many black fly species has been termed "Balbiani Ring" by Rothfels and Dunbar (1953).

In S. damnosum, this marker characterizes chromosome II and is located in section 53 just below the "double bubble" from which it is separated by three dark staining bands. Both landmarks hardly stain and though both usually "explode" the "Balbiani Ring" is generally larger and more "glassy".

The expression of the "Balbiani Ring" is variable. In Plate 5, Fig. 26, it is expressed as a "puff" composed of a tight aggregation of bands; in Fig.30, this structure appears to have decomposed into a cottony mass in which only a few bands are observable. The "exploded" condition in which no bands but "glassy" fibres are observed is shown in Plate 6, Fig.36. This and the intermediate condition shown in Fig. 32 came from the same individual.

"Para Balbiani"

In addition to the "Balbiani Ring", chromosome II is further characterized by a "glassy puff" in section 61. This landmark has been named the "Para Balbiani" because it is similar to that described by Basrur (1959) in Prosimulium inflatum.

Paracentric inversions resulting in shifts in the positions of the "Para Balbiani" are known from "Bandama" and "Soubre" in the "Nile" subgroup (Plate 5, Figs. 28, 30). In the "Sanje",subgroup Dunbar (1969) has reported a similar observation in "Nkusi".

"Blister"

This landmark is located in 76A of the short arm of chromosome III and is homologous to that reported by Rothfels (1956) in the Prosimulium species group.

The "blister" is recognized as a "puff" as shown in Plate 7, Fig.40.

DESCRIPTION OF STANDARD CHROMOSOMES

Standard Chromosome I

Standard chromosome I is shown in Plate 3, Fig.12 and Plate 4, Fig.19. It is characterized by an expanded region, section 21, in which the centromere is located. This is the largest of the three chromosomes and contains 42 sections. It is metacentric for the arm ratios about the centromere are approximately 1:1.1.

The short arm is recognized by the nucleolar site in section 19. The IS end is usually flared and consists of about six light staining bands in section IAB. A dark concave band in IC is a ready marker of the IS end.

Following this key band is a light one and a constriction which marks the start of section 2 in which all the bands are usually dark, compact, and easily resolved; the end of the section shows a unique light area. Except for the double band in 3A, this section is usually singly banded and stains moderately. Section 4 is characterized by two double bands which enclose a light staining region in the centre of which is a thin but well resolved band.

Subsection 6A is characteristically light, consequently the bands therein are always just visible. This is shown in Plate 3, Fig. 12. where the adjacent dark staining pair of bands in 6BC can also be seen. Behind these and up to section 10 are 5 groups of dark bands separated by light staining regions. In section 12AB is the characteristic "capsule".

Section 13C and 14 stand out conspicuously as a bulbous, light group. It is supported proximally by 13B, a solid double band and distally by a belt of three bands in 14C. From there to 18AB which borders the nucleolar site, one observes a preponderance of dark bands. The most outstanding of these are those in 15A with serrated edges. In contrast, the adjacent expanded region in section 21 is typically heavily stained.

Standard IL arm is shown in Plate 4, Fig. 19. The first section 22, adjacent to the expanded region consists of four moderately stained double bands. Section 23A is characteristically light. Other

landmarks of IL include sections 28 & 32. Section 28B comprises two dark double bands between which is an aggregation of light bands. Section 32 consists of three dark double bands. The light area shared by Section 32 and 33 is often stretched and provides a useful marker of the arm as shown by interspecific homologies in Plate 4. Another marker is provided by 35A, a conspicuous, heavy, dark band. The heavy double groups in 38A are a unique feature of the IL arm.

Standard Chromosome II

Standard chromosome II is shown in Plate 5, Fig. 26. The easily observed features of this chromosome are the "double Bubble" in 51C and 52A, the "Balbiani Ring" in section 53 and the "Para Balbiani" in section 61. The centromere is located in section 55. Chromosome II is submetacentric, the arm ratios about the centromere being approximately 1:1.5.

The tip of the IIS arm is characterized by a pair of moderately staining bands in 43BC. A light area in 43C separates this group from two double dark bands in 44A; 44 BC consists of a double band and a light area which merges with a sharp dark band followed by a heavy dark double one in 45B.

Section 46 consists of four sharp double bands separated by single lighter bands. The last double band is connected to the heavy doubles of 47A by a fibrous strand. The rest of section 47 stains

moderately, the remaining sections leading to the "double bubble" stain heavily. The "Ring of Balbiani" is separated from the "double bubble" by a constriction of three double bands and from the centromere by three heavy staining bands followed by a light region of fine bands.

The centromere end of the IIL arm shows two consecutive relatively light sections except for 56CI and 57AI which together constitute two heavy double bands. These double bands lie in a constricted region that is typical of this arm. A clear area in 57B connects with 57C and 58A each of which contains a double band. The rest of 58 is light except for a sharp, dark, concave band in 58C.

Behind the "Para Balbiani" are two dark staining sections. Directly in front of the "Para Balbiani" is a light area with two fine bands plus a darker double one. In 62A is a pair of three double light bands with a darker pair in the centre. The lighter outer pairs plus 62B can just be seen while 63AB stands out more prominently. Except for one double band in 63C, this region is typically light and thin, the bands therein being reduced to mere impressions. In general, therefore, sections 62 and 63 constitute a light zone characterized by four central, resolvable bands. As shown in Plate 5, Fig.26, sections 65, 66, and 67 constitute another landmark for the IIL arm. Starting from 65, there is a sharp dark double band followed by another double but convex one. The next group forms a "bump" of three closely united double bands that stain deeply. An area of light bands marks the

beginning of section 66. In 66AB are a group of four bands symmetrically arranged. 66C and 67AB together comprise a conspicuous dark series in which two double bands are flanked on each side by one sharp one. Section 68 consists of three evenly spaced double bands in which the central pair is thickest. The next section is similarly constituted except that there is a light region between the double band in 69A and the doubles in 69BC. In the latter 69BC is heavier and stains darker. Section 70 is characteristically bulged. It is a collection of mainly three double bands with fine light ones in between. Of the doubles, that in 70A is larger and usually darker. This group together with the dark three double bands in 71AB and the two dark heavy bands in 72B characterize the proximal end of the IIL arm. The tip of the arm is flared.

Standard Chromosome III

Standard chromosome III is shown in Plate 7, Fig. 37. It is submetacentric and is the shortest of the three chromosomes. The centromere is located in section 82 and the arm ratios about it are approximately 1:1.8.

The tip of the IIIS arm is typically fan shaped and light staining except for one dark double band in 74A. Two very heavy double bands in 75BC mark the start of the region named the "blister". In 77A are a thin light group followed immediately by a heavy double one. An area of light staining bands constitutes 77B which is supported

below by two dark double bands in 77C. Excepting one dark sharp band in 81B, this section is generally light staining. Below it is the large centromere.

Starting from the centromere end, the IIIL arm shows two sharp double bands in 82C and 83B. The remaining bands in these sections are light. In 85C are two double bands that are dark staining. A symmetrical association of bands is shown by 87BC and 88AB; section 89 comprises a similar group. A series of bands within sections 94-96 shows a constant expression and therefore forms key landmarks for the IIIL arm. There are two dark double bands in 94B. Following these in section 95 is a light region of faint bands, a prominent single band, a dark double band and a group of three heavy, dark staining bands.

CHAPTER 3

CYTOLOGICAL ANALYSIS OF "BILLE-YAH" SIBLINGS OF S. DAMNOSUM

Seven sibling species were identified in this study: tentatively these are called "Bille", "Yah", "Bandama", "Soubre", "Nile", "Sirba", and "Dieguera" according to the name of the locality where each was initially identified.

On the basis of shared interspecific inversions and inversion polymorphisms held in common, these species constitute three main categories: "Bille-Yah", "Bandama -Soubre" and "Nile-Sirba-Dieguera". Table 1 shows a summary of inversion differences between species. These are also shown in Plates 2 to 7.

"Bille-Yah" Siblings

"Bille" and "Yah" have the closest chromosomal pattern to "Nyamagasani" from which they differ by IS-1 and IL-3 (Plate 3, Figs. 12, 13, 14).

The two siblings differ from each other in that in "Bille" sex differentiation is based on C_I, while in "Yah" a rearrangement in the IIL arm, IIL-18, is sex linked; it is autosomal in "Bille".

"Bille" and "Yah" share IIS-6 but the remaining inversion polymorphisms provide additional basis of distinction: IS-2, IS-11, IL-15, IIS-5 and IIIL-16 are restricted to "Bille" while IL-12 and IIL-40 are restricted to "Yah".

Micro "morphological" characters namely; large, dark-staining centromeres, ectopic pairing of the centromeres and centric fusion found exclusively in "Yah" are also excellent markers.

"Yah" appears to breed exclusively in small shallow creeks in the forest region while "Bille" is known from rivers or their tributaries in both the Savannah and forest regions. The two species have never been found sympatrically, though the sympatry of each with other species is known. Each of these features will be treated in more detail starting with sex determination.

Sex Determination in "Bille" and "Yah"

In many black fly species X and Y chromosomes are indistinguishable (X_0Y_0) and sex determination is therefore assumed to differ at the level of the gene.

In other species of black flies X and Y chromosomes are characterized by differential chromosome segments usually relatively inverted and observed heterozygously in males and homozygously in females as reported by Rothfels (1956) in species of Prosimulium.

This led the same author to suggest that "it is a priori improbable that an inversion as such is the primary sex determining mechanism..." Since then observations on sex determination in P. multidentatum and P. magnum by Ottonen and Nambiar (1969), in P. mixtum and P. fuscum by Rothfels (1973) support this view.

Findings in "Bille" and "Yah" further illustrate these principles.

Sex determination in "Bille" is based on chromosome I and involves the expanded region C_I. In "Bille" a total of 89 sexed males were examined. Non-pairing of the expanded region of chromosome I was found in 41 males and 3 specimens of undetermined sex (Table 3). One homologue of these sections was standard, the other differentiated (Plate 4, Fig. 21).

The differential segment differs from the standard arrangement by having a slightly larger expanded region and by lacking the dark-staining bands characteristic of the standard sequence. In contrast all 127 females, the remaining 48 males and 61 individuals of undetermined sex were homozygous for the standard segment and showed complete pairing of homologous bands (Plate 4, Fig. 20).

Since the differential segment is exclusive to about half of the males, this segment is interpreted as marking a genetic Y chromosome (Y_1).

The fact that about half the males lack this cytological marker and therefore appear identical with females indicates the presence of a second Y chromosome which prevails as an ancestral, undifferentiated chromosome, indistinguishable from the X. It is therefore designated as Y_0.

As regards sex and IIL-18 in "Bille" (Table 4), in a total of 280 specimens, 87 males, 122 females and 58 individuals of undetermined sex were standard in this region, 2 males, 5 females, and 6 specimens of undetermined sex were heterozygous for IIL-18. In "Bille" therefore there was no evidence of association of IIL-18 and sex, although theoretically, the possibility that IIL-18 marks a rare X is not excluded.

In "Yah" on the other hand, IIL-18 is clearly associated with sex. Details are shown in Table 4. A total of 1179 specimens were examined; 915 individuals were homozygous for IIL-18; of these 154 were unsexed; 264 were heterozygous; of these 35 were unsexed. The standard sequence was never found homozygously.

Among the sexed individuals that showed heterozygosity for IIL-18, were 215 males, and 14 females. The large preponderance of male heterozygosity indicates the presence of a Y chromosome or male heterogamety, the 14 females being exceptions. Female homogamety was therefore expected. If this in fact exists one would expect all females but no males to show homozygosity. Contrary to expectation 252 males and 509 females homozygous for IIL-18 were found.

Thus while almost all females as expected were homozygous for IIL-18 so were a substantial number of males. This finding then suggests the presence of a Y chromosome, IIL-18, alternate to standard and identical with the X chromosome. It is therefore designated as Y_1. Speculations on the evolutionary relationships of these various sex chromosomes are given in the discussion.

What remains to be discussed is the nature of the 14 exceptional S/IIL-18 females. Three possibilities present themselves:

(1) error in sexing larvae, that is, the presumed females were in fact males.

(2) persistence of a rare, ancestral X chromosome of the standard sequence (X_0).

(3) the exceptional females may be due to crossing over in S/IIL-18 males.

The concept that sex associated inversions are probably sex modifiers admits the possibility that the sex locus lies not within the inversion but outside it. The sex locus would therefore be separated from the inversion by a homologous segment.

If in a male "Yah" crossing over occurred in this homologous segment, a crossover X chromosome (COX) with the standard sequence would be produced. The 14 females heterozygous for IIL-18 in "Yah" could be interpreted on this basis and this is the interpretation preferred by the writer.

In view of the total number of "Yah" females studied, 14 represents a relatively low number for cross-over types. Perhaps such females are selected against: the presence of male modifiers carried in the standard sequence might jeopardize normal development and reproductivity.

One might expect as well to recover the reciprocal crossover product (COY with IIL-18). This may in fact occur but how frequent it is cannot be estimated because a large proportion of Y chromosomes carry IIL-18 inherently.

Ectopic Pairing of Centromeres and Centric fusion

Ectopic pairing of centromeres (Plate 1, Fig. 4) was observed so frequently in "Yah" that this feature and the characteristic, large dark-staining centromere especially became ready markers by which "Yah" could be recognized at a glance. Large, dark centromeres were found consistently in all "Yah" individuals. Ectopic pairing was frequent but was not observed in the chromosomes of all nuclei of any specimen. This was thought to be due to the dissociating effects of pressure applied during chromosome preparation. Centric fusion (Plate 1, Fig. 3) noticeable by its hard, "glassy" appearance was also observed in "Yah" but was rare.

Inversion Polymorphisms in "Bille" and "Yah"

Floating inversions in "Bille" and "Yah" are summarized in Table 2, and shown in Plate 3, Figs. 13, 14; Plate 4, Fig.22; Plate 5, Fig. 27, Plate 7, Fig. 37. Where practical, samples were tested for Hardy-Weinberg conformity and all obeyed. Because these species were identified from collections from different geographic regions, the Table is arranged in accordance with geographic zones to show collection sites

and the distribution of inversions of "Bille" from Cameroon and Upper Volta, and of "Yah" from Liberia and Ivory Coast. Except for IIS-6 chromosomal polymorphism is clearly segregated in the "Bille" and "Yah". Within "Bille" there is a further restriction of floating inversions between Cameroonian and Voltaic populations. IS-2 IS-11, IIL-18, IIIS-5 and IIIL-16 are all exclusive to Cameroonian "Bille".

IS-2 : This is the most frequent of all. It was recorded from seven of the ten "Bille" localities: two sites on the Blackwater River, one on the Vina River and four on the Bille River. both heterozygous and homozygous alternates of IS-2 were found from two collections from the Bille River; the frequency of the inverted constituent being 0.15. The incidence of IS-2 is clearly heterogeneous as shown by 2X3 homogeneity tests. For samples #101 and 104 ($P < 0.0001$) in "Bille" as well as collections #55 and 67 in "Yah".

IS-II: This inversion showed a frequency of 0.05 and was observed from five collections: four on the Bille River and the fifth on the Blackwater River.

IIL-18: A frequency of 0.06 was observed for this inversion. It is known from two sites on each of Blackwater and Bille Rivers.

IIIS-5 and IIIL-16: These inversions are rare; each was observed from the Bille river and in each case from one collection. Their frequencies are 0.01 and 0.005 respectively.

IL-15 "Bille" from Upper Volta appears less polymorphic than "Bille" from Cameroon for only a single inversion, IL-15 was observed. This inversion is recorded from three of the four Voltaic sites and shows a frequency of 0.05. "Bille" on the whole has a wider spectrum of floating inversions than "Yah" (Table 2).

IL-12 and IIL-40: In "Yah" two floating inversions, IL-12 and IIL-40 exclusive to this species were observed; of these IL-12 is more widely spread; it occurs in ten of the thirty-one "Yah" sites located on seven river systems (Table 2). Among these rivers IL-12 was recorded from two out of six sites from St. Paul and Lofa where the frequencies are 0.05 and 0.02 respectively. The next incidence of note came from two of the three sites on the Cestos. Here the frequency of IL-12 is 0.04. Of the 156 individuals from the Farmington only two had this inversion. The same observation was made at Bendaja and Gbarpi creeks where 70 specimens were analysed. There were no records of IL-12 from both the Cavally and St. John from where 242 specimens were analysed.

The distribution of IIL-40 in "Yah" is even more limited. It is on record from one collection from the St. Paul and two from the Lofa (Table 2). In the former location 4 out of 236, in the latter 6 out of 185 had the inversion.

IIS-6: This single inversion shared by "Yah" and "Bille" is clearly more frequent in "Yah". In "Bille" it is restricted to the

Cameroon population where it is known from two collections; one from the Blackwater and the other from the Bille. In these locations a total of 203 specimens were analyzed. IIS-6 was present in only two.

In "Yah" IIS-6 is known from all but 12 of the 31 collections of the species and has been observed from all seven major watersheds inhabited by "Yah". (Table 2).

In two of these, St. Paul and the Cestos, both the heterozygous and homozygous sequences of this inversion were observed.

The frequencies of IIS-6 are 0.14, 0.14, 0.29, 0.1, 0.01, 0.02 0.08, and 0.06 for St. Paul, Lofa, Cestos, Cavally, St. John, Farmington and Bendaja-Gbarpi creeks respectively.

Distribution of "Bille" and "Yah"

(Map 1 shows the distribution of "Bille" and "Yah"). "Bille" has been recorded from Upper Volta in the northwestern side of West Africa and from Cameroon in the south eastern areas. In Upper Volta "Bille" is known from tributaries of the Black Volta River at Guena, Chute Dienkoa, Lanviera and Banzo, all in the Savannah zone. In the last three localities it was found sympatric with "Nile" and "Sirba".

From Cameroon on the other side of West Africa, "Bille" is known to be the sole sibling of S. damnosum from ten locations : four from the Blackwater River, five from the Bille River where this species was initially discovered, and one from Vina, a tributary of the

Sanga. Collections from the Blackwater and Bille Rivers all came from locations very close to the mountainous, forested Kumba area in Western Cameroon. Therefore except for one location on Vina River in the Guinea Savannah, "Bille" from Cameroon is known from the forest region and in hilly country.

In both forest and Savannah areas where "Bille" has been identified, "typical forest" transmission of onchocerciasis has been reported by Duke (1966) in Cameroon and by Philippon (1971, 1973, personal communication) in Upper Volta.

"Yah" was initially identified from Yah Creek in Liberia; here, within the forest zone, "Yah" is recorded from numerous other locations (Table2) all in the small shallow tributaries of the Lofa, Cestos, Farmington, St. John, and St. Paul Rivers. Garms (1973) has observed that in Liberia, this species shows a strong preference for shady habitats. A similar situation prevails in Western Ivory Coast where "Yah" is known from six locations : three from the tributaries of the Cavally River and three from those of the Sassandra River. The distribution of "Yah" along the Sassandra is known from streams at Zordo near Issia, Danane-Man Road near Danane and Zozola, a few miles from Soubre. At Zozola, hybridization between "Yah" and Bandama" was observed in two specimens. The distribution of "Yah" along the Cavally River is known from a tributary at Tai, another at Oua and the third at Toulepleu. At Toulepleu, "Yah" is sympatric with "Bandama", "Nile" and "Sirba".

"Yah" is considered anthropophilic in Liberia by Garms (1973). In Ivory Coast, Philippon (1971, 1973, personal communication) has reported "typical forest" transmission of onchocerciasis at Oua, a known Yah area, but has in contrast observed "zoophilic females" at similar areas at Tai and Danane Man Road. Therefore further investigations are required to understand the biting habits of "Yah".

TABLE 1

Summary of Inversion Differences between Species

Species	IS	IL	IIS	IIL	IIIS	IIIL
"Nyamagasani"	S/S	S/S	S/S	S/S	S/S	S/S
"Bille"	IS-1	IL-3	-*	-	-	-
"Yah"	IS-1	IL-3	-	IIL-18	-	-
"Soubre"	IS-1	IL-3.6	-	IIL-4.6	-	IIIL-2.17+4
"Bandama"	IS-1	IL-3.6	-	IIL-4.6,7	-	IIIL-2.17+4
"Nile"	IS-1	IL-1+3	-	IIL-C	-	** IIIL-2, IIIL-2.6
"Sirba"	IS-1	IL-1+3	-	IIL-C.8	-	** IIIL-2.6, IIIL-2
"Dieguera"	IS-1, IS-2	IL-3.12	-	IIL-C.8	-	IIIL-2

* represents standard arrangement
** shown in descending order of frequencies

TABLE - 2 : Inversion Polymorphisms in "Bille" And "Yah"

1 of

TABLE 2

INVERSION POLYMORPHISMS IN "BILLE AND "YAH"

		BILLE												
		Cameroon											Upper V	
Location*		Black water River			Vina River		Bille River						Black Volta River	
Sample #		96	97	98	99	100	101	102	103	104	105	Total	16	17	18
Total Larvae		15	1	12	7	5	21	18	38	71	15	203	8	60	3
	S/S	15	1	10	5	1	16	18	38	71	15	190	8	60	3
IIL-18	S/18	-	-	2	2	4	5	-	-	-	-	13	-	-	-
	18/18	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C_I	S/S	12	1	12	5	4	19	13	31	63	12	172	5	50	3
	S/a**	3	-	-	2	1	2	5	7	8	3	31	3	10	-
	S/S	15	1	9	5	4	21	17	31	37	8	148			
IS-2	S/2	-	-	3	2	1	-	1	7	27	5	46			
	2/2	-	-	-	-	-	-	-	-	7	2	9			
	S/S	15	1	11	7	5	21	15	36	55	14	180			
IS-11	S/11	-	-	1	-	-	-	3	2	16	1	23			
	11/11	-	-	-	-	-	-	-	-	-	-	-			
	S/S	15	1	12	7	5	21	18	36	71	15	201			
IIIS-5	S/5	-	-	-	-	-	-	-	2	-	-	2			
	5/5	-	-	-	-	-	-	-	-	-	-	-			
	S/S	15	1	12	7	5	21	17	38	71	15	202			
IIL-16	S/16	-	-	-	-	-	-	1	-	-	-	1			
	16/16	-	-	-	-	-	-	-	-	-	-	-			
	S/S												7	48	3
IL-15	S/15	-	-	-	-	-	-	-	-	-	-	-	1	12	-
	15/15	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	S/S														
IL-12	S/12	-	-	-	-	-	-	-	-	-	-	-			
	12/12	-	-	-	-	-	-	-	-	-	-	-			
	S/S														
IIL-40	S/40	-	-	-	-	-	-	-	-	-	-	-			
	40/40	-	-	-	-	-	-	-	-	-	-	-			
	S/S	15	1	12	6	5	21	18	37	71	15	201	8	60	3
IIS-6	S/6	-	-	-	1	-	-	-	1	-	-	2	-	-	-
	6/6	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* For details refer to Table 10
** a represents differential segment
Where no entry is made standard is assumed

2 of

YAH

			Liberia Tributaries of St. Paul River							Liberia Tributaries of Lofa River							Liberia Tributaries of Cestos River			
di r	Total	Grand Total	80	81	82	83	84	85	Total	87	88	89	90	91	92	Total	53	54	55	Tot
	77	280	32	30	17	105	7	45	236	26	11	31	56	9	52	185	60	3	75	13
	77	267	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	-	13	9	11	4	37	1	14	76	1	-	11	16	-	5	33	3	-	6	
	-	-	23	19	13	68	6	31	160	25	11	20	40	9	47	152	57	3	69	12
	64	236	32	30	17	105	7	45	166	26	11	31	56	9	52	185	60	3	75	13
	13	44	-	-	-	-	-	-	70	-	-	-	-	-	-	-	-	-	-	
	62	252																		
	14	28																		
	-	-																		
			32	21	17	102	7	45	224	23	11	31	55	9	52	181	59	3	71	13
			-	9	-	3	-	-	12	3	-	-	1	-	-	4	1	-	4	
			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
			28	30	17	105	7	45	232	26	11	26	55	9	52	179				
			4	-	-	-	-	-	4	-	-	5	1	-	-	6				
			-	-	-	-	-	-	-	-	-	-	-	-	-	-				
	77	278	22	30	17	91	6	38	204	23	10	28	46	7	45	159	60	1	40	10
	-	2	9	-	-	14	1	7	31	3	1	3	10	2	7	26	-	2	28	3
	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	7	

3 of

			Ivory Coast Tributaries of Cavally River				Liberia Tributaries of St. John River					Liberia Tributaries of Farmington River					Ivory Coast Tributaries of Sassandra River				Liberia Bendaja Cree	
es of ver	55	Total	49	50	51	Total	61	62	63	64	Total	66	67	68	69	Total	46	47	48	Total	94	9
	75	138	26	1	109	136	15	19	23	49	106	1	105	45	7	158	94	2	54	150	55	1
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	6	9	-	-	8	8	6	8	3	18	35	-	48	18	-	67	1	-	1	2	29	
	69	129	26	1	101	128	9	11	20	31	71	1	57	26	7	91	93	2	53	148	26	1
	75	138	26	1	109	136	15	19	23	49	106	1	105	45	7	158	94	2	54	150	55	1
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	71	133	26	1	109	136	15	19	23	49	106	1	105	43	7	156	94	2	53	149	54	
-	4	5	-	-	-	-	-	-	-	-	-	-	-	2	-	2	-	-	1	1	1	
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
1	40	101	26	1	96	123	15	19	22	48	104	1	105	42	7	155	94	2	42	138	52	
2	28	30	-	-	13	13	-	-	1	1	2	-	-	3	-	3	-	-	12	12	3	
-	7	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Total
136
-
8
128
136
-
136
-
123
13

	Liberia Tributaries of St. John River					Liberia Tributaries of Farmington River					Ivory Coast Tributaries of Sassandra River				Liberia Bendaja-Gberpi Creeks			
Total	61	62	63	64	Total	66	67	68	69	Total	46	47	48	Total	94	95	Total	Grand Total
136	15	19	23	49	106	1	105	45	7	158	94	2	54	150	55	15	70	1179
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	6	8	3	18	35	-	48	18	-	67	1	-	1	2	29	5	34	264
128	9	11	20	31	71	1	57	26	7	91	93	2	53	148	26	10	36	915
136	15	19	23	49	106	1	105	45	7	158	94	2	54	150	55	15	70	1179
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
136	15	19	23	49	106	1	105	43	7	156	94	2	53	149	54	14	68	1153
-	-	-	-	-	-	-	-	2	-	2	-	-	1	1	1	1	2	26
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
123	15	19	22	48	104	1	105	42	7	155	94	2	42	138	52	14	66	1050
13	-	-	1	1	2	-	-	3	-	3	-	-	12	12	3	1	4	121
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8

TABLE - 3 : The Relation Between Sex and the Expanded Region of Chromosome I in "Bille"

1 of

TABLE 3

The Relation between Sex and the Expanded Region of Chromosome I in Bille

		Cameroon									
Location*		Blackwater River				Vina River			Bille River		
Sample #		96	97	98	99	100	101	102	103	104	105
Total larvae		15	1	12	7	5	21	18	38	71	15
C_1 **	♂	2	1	2	-	-	4	3	2	22	3
S/S = X_0Y_0	♀	2	-	5	5	1	4	4	20	37	3
	?	8	-	5	-	3	11	6	9	4	6
	♂	3	-	-	2	1	2	5	6	8	3
S/Y_1	♀	-	-	-	-	-	-	-	-	-	-
	?	-	-	-	-	-	-	-	1	-	-

* For details refer to Table 9
** Sex differential segment, refer to Plate 4, Fig, 21

egion of Chromosome I in Bille

Cameroon							Upper Volta					
Vina River	Bille River						Black Volta River		Plandi River			
100	101	102	103	104	105	Total	16	17	18	19	Total	Grand Total
5	21	18	38	71	15	203	8	60	3	6	77	280
-	4	3	2	22	3	39	-	6	-	3	9	48
1	4	4	20	37	3	81	1	42	2	1	46	127
3	11	6	9	4	6	52	4	2	1	2	9	61
1	2	5	6	8	3	30	3	8	-	-	11	41
-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	1	-	-	1	-	2	-	-	2	3

to Plate 4, Fig, 21

TABLE - 4 : The Relation Between IIL-18 and Sex in "Bille" and "Yah"

1 of

TABLE 4

The Relation between IIL-18 and sex in "Bille and Yah"

BILLE

		Cameroon										
Location*		Blackwater River				Vina River	Bille River					
Sample #		96	97	98	99	100	101	102	103	104	105	T
Total larvae		15	1	12	7	5	21	18	38	71	15	2
IIL-S/S	♂	5	1	2	2	1	4	8	8	30	6	
	♀	2	-	3	3	-	4	4	20	37	3	
	?	8	-	5	-	-	8	6	10	4	6	
IIL-S/18	♂	-	-	-	-	-	2	-	-	-	-	
	♀	-	-	2	2	1	-	-	-	-	-	
	?	-	-	-	-	3	3	-	-	-	-	
IIL-18/18	♂	-	-	-	-	-	-	-	-	-		
	♀	-	-	-	-	-	-	-	-	-	-	
	?	-	-	-	-	-	-	-	-	-	-	

* For details see Table 10

2 of 2

'Bille and Yah"

Cameroon								Upper Volta					
	Vina River	Bille River						Black Volta River			Blandi River		
99	100	101	102	103	104	105	Total	16	17	18	19	Total	Grand Total
7	5	21	18	38	71	15	203	8	60	3	6	77	280
2	1	4	8	8	30	6	67	3	14	-	3	20	97
3	-	4	4	20	37	3	76	1	42	2	1	46	122
-	-	8	6	10	4	6	47	4	4	1	2	11	58
-	-	2	-	-	-	-	2	-	-	-	-	-	2
2	1	-	-	-	-	-	5	-	-	-	-	-	5
-	3	3	-	-	-	-	6	-	-	-	-	-	6
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE - 4 : Continued

1 of

TABLE 4 (Cont'd)

YAH

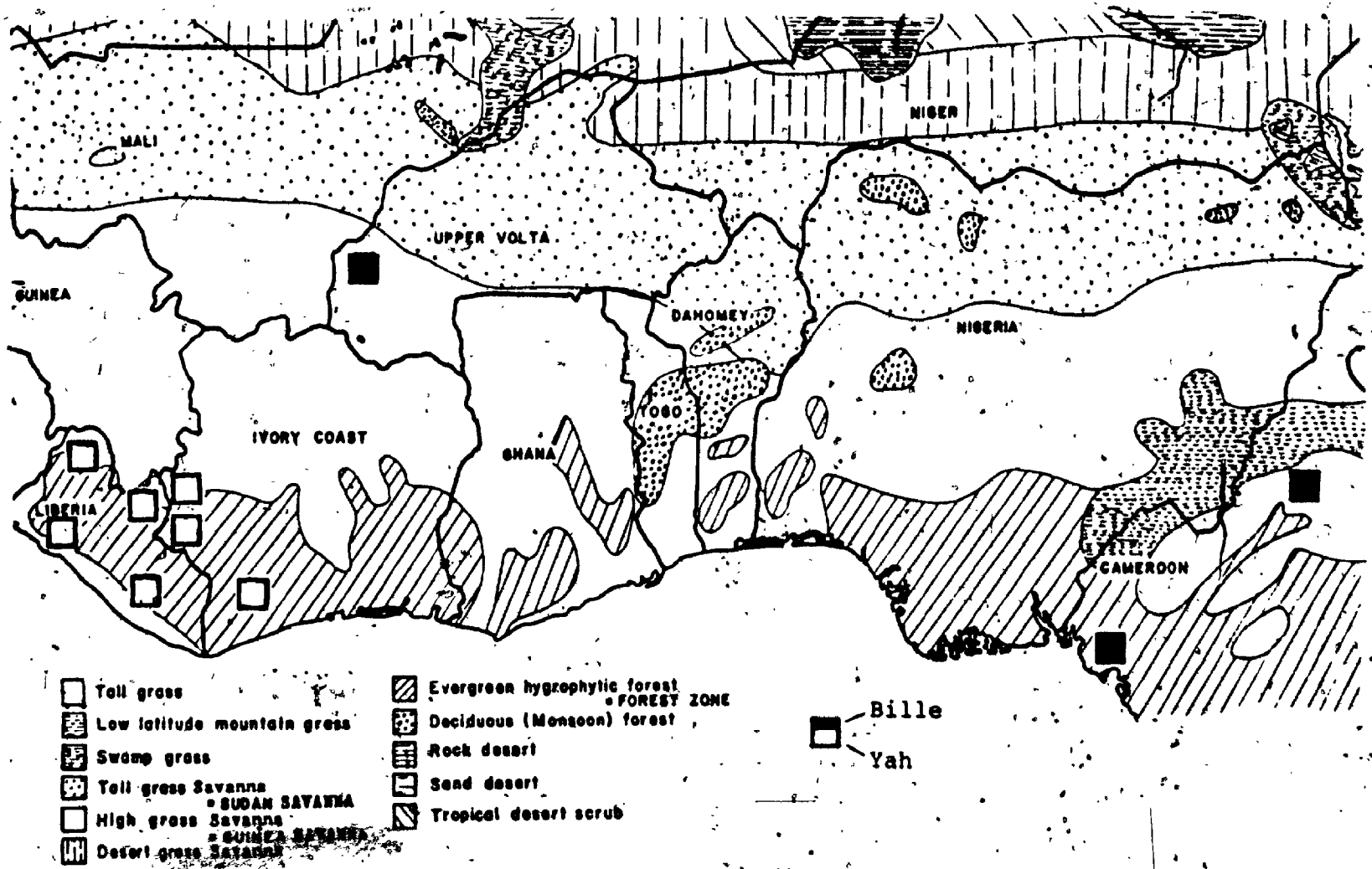
Location*		Liberia Tributaries of St. Paul River							Liberia Tributaries of Lofa				River	
Sample #		80	81	82	83	84	85	Total	87	88	89	90	91	92
Total larvae		32	30	17	105	7	45	236	26	11	31	56	9	52
IIL-S/S	♂	-	-	-	-	-	-	-	-	-	-	-	-	-
	♀	-	-	-	-	-	-	-	-	-	-	-	-	-
	?	-	-	-	-	-	-	-	-	-	-	-	-	-
IIL-S/18	♂	6	9	3	28	1	10	57	1	-	7	14	-	5
	♀	-	1	-	3	-	1	5	-	-	1	-	-	-
	?	3	1	1	6	-	3	14	-	-	3	2	-	-
IIL-18/18	♂	6	6	3	10	2	2	29	6	4	3	14	4	19
	♀	11	10	4	47	4	22	98	10	4	16	23	5	20
	?	6	3	6	11	-	7	33	9	3	1	3	-	8

	Liberia				Ivory Coast				Liberia					Tributa	
	Tributaries of Cestos River				Tributaries of Cavally River				Tributaries of St. John River					Riv	
Total	53	54	55	Total	49	50	51	Total	61	62	63	64	Total	66	67
185	60	3	75	138	26	1	109	136	15	19	23	49	106	1	105
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	3	-	5	8	-	-	6	6	4	6	3	15	28	-	42
1	-	-	1	1	-	-	-	-	2	1	-	3	6	-	-
5	-	-	-	-	-	-	2	2	-	1	-	-	1	-	6
50	18	-	11	29	12	1	53	66	-	-	5	1	6	-	-
78	29	1	42	72	11	-	37	48	8	7	13	27	55	1	44
24	10	2	16	28	3	-	11	14	1	4	2	3	10	-	13

3 of 3

	Liberia					Ivory Coast				Liberia			
ver	Tributaries of Famington River					Tributaries of Sassandra River				Bendaja- Gbarpi Creeks			
tal	66	67	68	69	Total	46	47	48	Total	94	95	Total	Grand Tota
6	1	105	45	7	158	94	2	54	150	55	15	70	1179
-	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	-	-	-
3	-	42	17	-	59	1	-	1	2	24	4	28	215
	-	-	-	-	-	-	-	-	-	-	1	1	14
	-	6	2	-	8	-	-	-	-	5	-	5	35
	-	-	5	3	8	36	-	20	56	6	2	8	252
	1	44	17	3	65	45	-	22	67	18	8	26	509
	-	13	4	1	18	12	2	11	25	2	-	2	154

MAP - 1 : The Distribution of "Bille" and "Yah"


MALI
NIGER
UPPER VOLTA
GUINEA
DAHOMEY
NIGERIA
TOGO
IVORY COAST
GHANA
LIBERIA
CAMEROON
Tall grass
Low latitude mountain grass
Swamp grass
Tall grass Savanna
• SUDAN SAVANNA
High grass Savanna
• GUINEA SAVANNA
Desert grass Savanna
Evergreen hygrophytic forest
• FOREST ZONE
Deciduous (Monsoon) forest
Rock desert
Sand desert
Tropical desert scrub
Bille
Yah

CHAPTER 4

CYTOLOGICAL ANALYSIS OF "BANDAMA" AND "SOUBRE" SIBLINGS

"Bandama-Soubre" Siblings

This pair differs from "Bille" by the fixed inversions IIL-4, IIL-6 and IIIL-4 as well as the virtually fixed IL-6 and IIL-2.17 (Plate 4, Fig 23; Plate 5, Figs. 28, 29, 30; Plate 7, Fig.40). The intermediate rearrangement, namely IIIL-2 occurs only rarely both heterozygously with IIIL-2.17 and homozygously..

While sharing in common those inversions fixed with respect to "Bille", "Bandama" and "Soubre" differ by a critical rearrangement in the IIL arm, IIL-6.7 and by restricted inversion polymorphism. "Soubre" is characterized by IIL-6. "Bandama" is defined by IIL-6.7. However, a few individuals in populations of both "Bandama" and "Soubre" were heterozygous for IIL-6.7. No sex chromosomes were identified in either "Bandama" or "Soubre".

Restricted to "Soubre" are three floating inversions: IIIL-24, IIIL-25 and IIIL-26. Exclusive to "Bandama" are IS-20, IS-21; IL-18, IIIL-5, IIIL-23 and IIIL-30 (Table 5).

On the basis of the rearrangements in the IIL arm, all collections were organized into three groups comprising samples of clean "Bandama", clean "Soubre", and samples with mixed individuals of "Bandama" and "Soubre" including specimens heterozygous for IIL-6.7 (Table 5).

In the next section, the distribution of "Bandama" and "Soubre" and their inversion polymorphisms will be presented.

Distribution of "Bandama" and "Soubre"

The distribution of this pair is summarised in Map 2.

"Bandama" is known from the Bandama Valley in Ivory Coast, River Cavally on the Ivory Coast/ Liberia border, and Rivers St.Paul, Farmington, Lofa and Mano in Liberia. In all collections from these locations only two individuals were found heterozygous for IIL-6.7. One of these came from a collection of 32 specimens from the Mano, the other from a sample of 117 individuals from the Cavally (Table 5).

"Soubre" was recorded from Bandama Valley in Ivory Coast, the Leraba Bridge on the Upper Volta/Ivory Coast border and the Cestos River in Liberia (Table 5).

Various mixed populations of "Bandama", "Soubre" and individuals heterozygous for IIL-6.7 came from Rivers Bandama and Sassandra in Ivory Coast and St. Paul River in Liberia (Table 5). "Bandama" and rare individuals heterozygous for IIL-6.7 were present in one collection from the Bandama and in another from the St. Paul. "Bandama" and "Soubre" but no heterozygotes were observed in two samples from the Bandama. Both siblings and the heterozygotes were found together in single collections from the Bandama and Sassandra Rivers,

These mixed populations therefore, excepting the one sample from the St. Paul River in Liberia, came from Ivory Coast.

Considering both clean and mixed collections of "Bandama-Soubre" from the Liberian forest zone, a great majority of specimens from the St. Paul River were "Bandama" to the exclusion of "Soubre" while those from the Cestos were entirely "Soubre". It appears therefore that populations of "Bandama" and "Soubre" in Liberia are allopatric.

In Ivory Coast on the other hand, both allopatry and sympatry (Table 5) were observed: 34 individuals from Niakaramandougou and 80 from the Lerba Bridge both in the Savannah region, consisted of only "Soubre"; in the two mosaic forest areas, 5 specimens from Beoumi were also clean "Soubre" while 86 from Ahouati were entirely Bandama except one which was heterozygous for IIL-6.7. In the forest zone itself, two cases of clear sympatry are known from two collections from the Bandama Valley at Tiassale where in one sample 88 individuals of "Bandama" and 6 of "Soubre" were observed; in the other collection the proportions were 13:7. The remaining four collections from this region showed either "Bandama", "Soubre" and individuals heterozygous for IIL-6.7 occurring together or an association of the heterozygote with either sibling (Table 5).

"Bandama", the heterozygote and "Soubre" were observed in the proportions of 23:31:11 in a collection on the Sassandra at Soubre; in another collection from the Bandama at Tiassale, the proportions were

18:2:1; two "Bandama" specimens and one heterozygote are also known from a sample in the Tissale vicinity. Conversely 3 "Soubre" individuals and 4 heterozygotes were observed from one sample on the Sassandra near Soubre.

The findings in Liberia as well as Ivory Coast of sporadic allopatric populations of "Bandama" and "Soubre" suggest that they are discrete. However, allopatry by indirectly inhibiting hybridization is a poor measure of distinction.

On the other hand there are known areas (Table 5) in which clean "Bandama" and "Soubre" populations are sympatric. Such a coexistence in which both populations have retained their identity is an indication of specific status. It seems therefore that clean populations of "Bandama" and "Soubre" constitute discrete sibling species especially if it is assumed that all individuals heterozygous for the critical IIL-6.7 represent relics of an ancestral population. On this assumption "Soubre" carried a "relic" IIL-6.7 and/or Bandama carries on "relic" IIL-6.

Such an interpretation could apply in a situation where the unexpected is indeed rare. In the present case, though in most samples individuals heterozygous for IIL-6.7 were rare, there was one collection from the Sassandra in which about 50% of the specimens were heterozygotes. It appears odd to find so many such individuals in one collection. However, it is theoretically possible that this might occur in a population that is small, isolated and therefore inbreeding.

In brief, "Bandama" and "Soubre" populations have been recorded in the tropical forest zones of Liberia and Ivory Coast. "Bandama" has never been identified beyond this zone; "Soubre" however, is on record at the Leraba Bridge, a high grass Savannah region of southern Upper Volta.

There is little information on the biology of these species. In Liberia, Garms (1973) has observed that "Soubre" larvae settle in masses on rocks in contrast to larvae of "Bandama" and other species which are usually found attached to vegetation; and that in areas where "Soubre" predominates, man has not been "conspicuously approached" by black flies. In Ivory Coast, Philippon (1971, 1973, personal communication) suggests that both "Soubre" and Bandama" are known in endemic areas of onchocerciasis. They are therefore implicated in the transmission of the disease.

Inversion Polymorphisms in "Bandama" and "Soubre"

Apart from a few shared floating inversions, chromosomal polymorphism in "Bandama" and "Soubre" appear segregated as summarized in Table 5 and shown in Plate 3, Fig.I5, Plate 4, Fig. 23, Plate 5, Fig.30; Plate 7, Fig.40.

Where practical, samples were tested for Hardy-Weinberg conformity and most obeyed. The exceptions were samples #86 with respect

to IS-5 (p=0.005) and IIIL-23 (p=0.02) in "Bandama"; and #9 with respect to IIIL-24 (p=0.02) in "Soubre".

Shared Floating Inversions:

IS-5 This inversion is known from 11 out of 15 collections of clean "Bandama" from Liberia and Ivory Coast. It was observed heterozygously from one sample on the Bandama Valley, eight from the St. Paul, one from the Mano and another from the Lofa. At the last location, IS-5 was also observed homozygously. For clean "Bandama" collections, a frequency of 0.15 was observed.

The inversion was not observed in clean "Soubre" populations. The only known records came from "Soubre" in mixed populations where IS-5 was observed heterozygously from one individual in a collection on the Bandama Valley at Tiassale. A similar observation was made from a collection on the Sassandra. A frequency of 0.15 was observed.

The incidence of IS-5 is heterogeneous in "Bandama" populations as shown by 2 x 3 homogeneity tests. For samples #52 and 70, 52 and 86, 70 and 86 the P value was less than 0.0001.

IL-6 In both "Bandama" and "Soubre" this inversion is known almost exclusively homozygously (Table 5). In a grand total of 944 specimens, heterozygosity was observed only in two individuals

of "Bandama"; one from St. Paul and the other from the Bandama Valley; the rest were homozygous for IL-6. This inversion is therefore almost fixed.

Consequently instead of the IL-3 of "Bille" and "Yah" the standard arrangement for the IL arm for "Bandama" and "Soubre" is considered to be IL-3.6.

IL-1 This inversion has been observed floating in a few specimens of "Bandama" and "Soubre".

In Bandama, IL-1 has been observed heterozygously, IL-1+3/IL-3.6, in a specimen from one collection on the Mano and in another on the Cavally.

In "Soubre" the inversion is known heterozygously in four individuals from Niakaramandougou and ten from the Leraba: The rearrangement, IL-1+3/IL-1+3, was observed in "Bandama" from one specimen in a collection at Mano and in another from a sample on the Cavally. The frequencies were 0.008 and 0.06 for "Bandama" and "Soubre" respectively.

IIS-7 This inversion is known from clean"Bandama" "Soubre" and mixed populations. The inversion "captures" the "double bubble" and the "Balbiani Ring" which consequently became transposed by a homozygous rearrangement.

In "Bandama" IIS-7 was observed heterozygously in two collections from the St. Paul, one from the Farmington and another from a mixed St. Paul sample.

In this sibling, a peculiar observation suggesting a highly inbred population was observed from the Farmington collection in which the majority were homozygous for IIS-7, a few showed heterozygosity and none was standard (Table 5).

In "Soubre" IIS-7 is known from one individual in a sample from the Cestos River. The frequencies were 0.07, 0.004 and 0.004 for "Bandama", mixed populations, and "Soubre" respectively.

IIL-4.39 This inversion was observed heterozygously in two "Bandama" individuals from a collection on the Farmington and homozygously in one "Soubre" individual from a collection on the Cestos.

The frequency was 0.004 in both.

IIIL-2.17 This inversion is known heterozygously from eight locations. One on the Bandama Valley, near Tiassale, two from St. Paul, one from Farmington, Lofa, Cavally near Nyaake and Cavally near Toulepleu.

Homozygously, IIIL-2.17 is known from a majority of "Bandama" and "Soubre" collections (Table 5).

The frequencies of the inversion in clean "Bandama" clean "Soubre" and mixed populations are 0.00, 1.0 and 1.0 respectively.

It therefore appears that this inversion is virtually fixed in the entire population.

Floating Inversions restricted to "Bandama"

IS-20 This inversion is rare. It is known heterozygously from two collections, one on the Cavally at Toulepleu and the other at the Mano River. Four specimens from Mano and one from Toulepleu had this inversion. A frequency of 0.01 was observed.

IS-21 This inversion too is rare. It is known heterozygously from four individuals in a collection from the Farmington River. A frequency of 0.01 was observed.

IIIL-30 This is the most infrequent of all floating inversions observed in "Bandama". It is known heterozygously from one specimen in a Farmington sample. A frequency of 0.002 was observed.

IIIL-5 This inversion is known in both clean "Bandama" and in "Bandama" associated with mixed populations. In pure "Bandama"

IIIL-5 was recorded heterozygously from four locations: one from the "Bandama" Valley and three from the St. Paul River.

In the mixed populations IIIL-5 is known heterozygously and homozygously from two collections on the Bandama Valley near Tiassale.

In another sample from the Tiassale area, IIIL-5 was observed heterozygously in a "Bandama" specimen and an individual heterozygous for IIL-6.7. Heterozygosity for IIIL-5 was also observed in a specimen from the St. Paul.

The frequency in clean "Bandama" was 0.05, in mixed populations 0.18.

IIIL-23 This inversion is known from only clean "Bandama" collections.

Within "Bandama" IIIL-23 was recorded exclusively from Liberian samples on the Cavally, St. Paul, Lofa, Farmington and Mano Rivers. Apart from a collection from the Farmington St. Paul and Cavally in which IIIL-23 is known heterozygously only, the rest of the sites show this inversion heterozygously and homozygously. A frequency of 0.21 was observed.

From the observed frequencies of IIIL-5 and IIIL-23 assuming independent assortment, individuals in which both rearrangements

occur were expected. However, no such specimens were found. Perhaps, the geographical distribution of these rearrangements may explain this peculiar observation. IIIL-5 appears restricted to the Bandama Valley in Ivory Coast at Tiassale about 600 km from the nearest locations on St. Paul, Farmington and Lofa rivers in Liberia where IIIL-23 seems to be endemic It appears therefore that IIIL-5 and IIIL-23 are endemic in different areas.

Floating inversions restricted to "Soubre"

IIIL-24 This inversion is known from three collections all within Ivory Coast. It was recorded heterozygously from one sample at Beoumi and another at Niakaramandougou. At Leraba Bridge, IIIL-24 was observed homozygously as well. A frequency of 0.42was recorded.

The incidence of IIIL-24 is heterogeneous as shown by 2 x 3 homogeneity tests. For samples #9 and 14 the P value was 0.003. The absence of IIIL-24 in #56 and 58 indicates that they are significantly different from both #9 and #14.

IIIL-25 This inversion was observed heterozygously from three collections from the Cestos River. A frequency of 0.04 was observed.

IIIL-26 This inversion is known heterozygously from two samples on the Cestos River. A frequency of 0.03 was observed.

In all specimens except one, no combinations of these rearrangements were observed in an individual. IIIL-25/ IIIL-26 was recorded from one individual in a collection from the Cestos. Except for this single instance, the situation in "Soubre" as regards the endemicity of some rearrangements is similar to that presented from "Bandama".

Hybridization between "Yah" and "Bandama"

Hybridization between "Yah" and "Bandama" was observed in two individuals from Zozola stream, near Soubre-Sassandra in West Ivory Coast (Plate 8, Fig. 43). These hybrids were recognized by the presence of heterozygous configurations formed by the critical inversions which distinguish the two species and by the retention of centric ectopic pairing by the chromosomes of one parent, "Yah"; Plate 8, Fig. 44 shows the above features of the hybrid chromosomes.

Based on "Bille", "Yah" and "Bandama" are two steps apart in the IIL-arm. This difference is represented in the hybrid by the complex inversion, IIL-18/IIL-4.67. Another complex configuration is seen in the hybrid IIIL arm in which "Yah" is standard as opposed to "Bandama's" common homozygous sequence, IIIL-2.17+4. The simple inversion loops in the IS and IL arms respectively represent IS-5 and IL-6 which are also characteristic of "Bandama".

TABLE - 5 : Inversion Polymorphisms in "Bandama" and "Soubre"

TABLE 5

Inversion Polymorphisms in "Bandama" and "Soubre"

Location*		BV	CAV	St.P	St.P	St.P	St.P	St.P	St.p	BANDAMA St.P	St.P	St.P
Sample #		4	52	70	71	72	73	74	75	76	77	78
Total larvae		86	117	54	7	9	30	20	10	2	5	9
	** 6/6	-	-	-	-	-	-	-	-	-	-	-
IIL-6.7	6/6.7	-	1	-	-	-	-	-	-	-	-	-
	6.7/6.7	86	116	54	7	9	30	20	10	2	5	9
	S/S	85	117	41	6	7	25	16	9	2	4	8
IS-5	S/5	1	-	13	1	2	5	4	1	-	1	1
	5/5	-	-	-	-	-	-	-	-	-	-	-
	3/3	-	-	-	-	-	-	-	-	-	-	-
IL-6	3/3.6	-	-	-	-	-	-	-	-	-	-	-
	3.6/3.6	86	117	54	7	9	30	20	10	2	5	9
	3.6/3.6	86	117	54	7	9	30	20	10	2	5	9
IL-1	3.6/1+3	-	-	-	-	-	-	-	-	-	-	-
	1+3/1+3	-	-	-	-	-	-	-	-	-	-	-
	S/S	86	117	54	7	9	29	19	10			
IIS-7	S/7	-	-	-	-	-	1	1	-			
	7/7	-	-	-	-	-	-	-	-			
	2/2	-	1	-	-	-	-	-	-	-	-	-
IIIL-2	2/2.17+4	-	5	1	-	-	-	-	-	-	-	-
	2.17+4									-	-	-
	2.17+4	86	111	53	7	9	30	20	10	2	5	9
	S/S											
IS-20	S/20											
	20/20											
	S/S											
IS-21	S/21											
	21/21											
	S/S											
IIL-39	S/39											
	39/39											
	S/S	67							9	-	4	7
IIIL-5	S/5	19							1	-	1	2
	5/5	-	-	-	-	-	-	-	-	-	-	-
	S/S	86	116	54	5	4	14	18	9			
IIIL-23	S/23	-	1	-	1	2	8	2	-			
	23/23	-	-	-	1	3	8	-	-			
	S/S											
IIIL-24	S/24											
	24/24											
	S/S											
IIIL-25	S/25											
	25/25											
	S/S											
IIIL-26	S/26											
	26/26											
	S/S											
IIIL-30	S/30											
	30/30											
IL-18	S/18											

* In each c
** For deta
*** IIIL-25
Where no e

2 of

MIXED POPULATION

St.P	St.P	FAR	LOF	MAN	CAV	Total	BV	BV	BV	BV	St.P
77	78	65	86	93	50		1	3	5	2	79
5	9	32	48	32	2	463	21	3	94	20	38
-	-	-	-	-	-	-	1	-	6	7	-
-	-	3	-	1	-	2	2	1	-	-	1
5	9	32	48	31	2	461	18	2	88	13	37
4	8	32	14	25	2	393	21	3	94	19	11
1	1	-	15	7	-	51	-	-	-	1	27
-	-	-	19	-	-	19	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	1	-	-	-	1
5	9	32	48	30	-	459	20	3	94	20	37
5	9	32	48	30	-	459	20	3	94	20	37
-	-	-	-	1	1	2	-	-	-	-	-
-	-	-	-	1	1	2	-	-	-	-	-
		-									37
		5									1
		27									-
-	-	-	-	-	1	2	-	-	-	-	-
-	-	2	1	2	1	12	-	-	2	-	1
5	9	30	47	30	-	449	21	3	92	20	37
				28	1						
				4	1						
				-	-						
		28									
		4									
		-									
		30									
		2									
		-									
4	7						9	1	65	62	37
1	2						11	2	25	-	1
-	-						1	-	4	-	-
		25	8	10	-						
		7	14	10							
		-	26	12							
		31									
		1									

SA	SA	Total	BV
44	45		7
65	7	248	5
11	3	28	5
31	4	39	-
23	-	181	-
64	7	219	5
1	-	29	-
-	-	-	-
-	-	-	-
-	-	2	-
65	7	246	5
65	7	246	5
-	-	-	-
-	-	-	-
-	-	-	-
-	-	3	-
55	7	245	5
65	7	204	
-	-	39	
-	-	5	
			3
			2
			-
			5
			-
			1

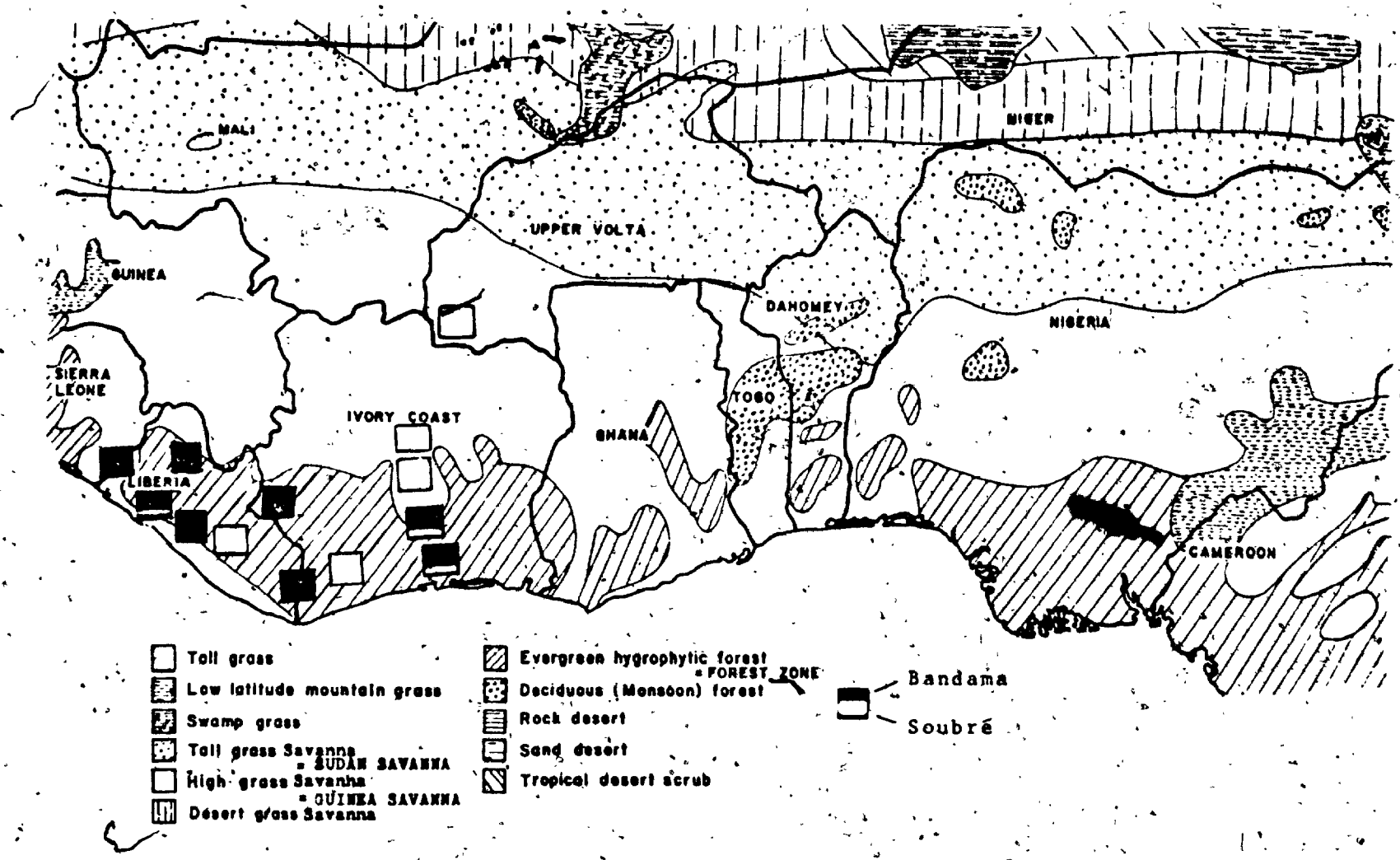
* In each of the zygote sequences the first is considered standard
** For details refer to Table 9
*** IIIL-25/IIIL-26 observed in one specimen (not shown)
Where no entry is made, standard is assumed

MIXED POPULATION						SOUBRE							
otal	BV	BV	BV	BV	St.P	SA	SA	Total	BV	BV	CES	CES	CES	LER	Total
	1	3	5	2	79	44	45		7	9	56	57	58	14	
63	21	3	94	20	38	65	7	248	5	34	73	7	34	80	233
-	1	-	6	7	-	11	3	28	5	34	73	7	34	80	233
2	2	1	-	-	1	31	4	39	-	-	-	-	-	-	-
61	18	2	88	13	37	23	-	181	-	-	-	-	-	-	-
93	21	3	94	19	11	64	7	219	5	34	73	7	34	80	233
51	-	-	-	1	27	1	-	29	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
-	1	-	-	-	1										
69	20	3	94	20	37	65	7	246	5	34	73	7	34	70	223
69	20	3	94	20	37	65	7	246	5	30	73	7	34	70	219
2	-	-	-	-	-	-	-	-	-	4	-	-	-	10	14
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
					37								33		
					1								1		
													-		
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	2	-	1	-	-	3	-	-	-	-	-	-	-
9	21	3	92	20	37	55	7	245	5	34	73	7	34	80	233
													33		
													-		
													1		
	9	1	65	62	37	65	7	204							
	11	2	25	-	1	-	-	39							
	1	-	4	-	-	-	-	5							
									3	17	73	7	34	29	163
									2	17	-	-	-	34	53
									-	-				17	17
									5	34	71	4	29	80	223
									-	-	2	3	5	-	10
									-	-	-		-	-	-
											69		31		
											4		3***		
											-		-		

nsidered standard

t shown]

MAP - 2 : The Distribution of "Bandama" and "Soubre"


MALI
NIGER
UPPER VOLTA
GUINEA
DAHOMEY
NIGERIA
SIERRA LEONE
IVORY COAST
GHANA
TOGO
LIBERIA
CAMEROON
Tall grass
Low latitude mountain grass
Swamp grass
Tall grass Savanna
High grass Savanna
Desert grass Savanna
SUDAN SAVANNA
GUINEA SAVANNA
Evergreen hygrophytic forest
Deciduous (Monsoon) forest
Rock desert
Sand desert
Tropical desert scrub
FOREST ZONE
Bandama
Soubré

CHAPTER 5.

CYTOLOGICAL ANALYSIS OF "NILE-SIRBA-DIEGUERA" SIBLINGS

"Nile-Sirba" Siblings

"Nile" and "Sirba" differ from "Bille" by IL-1 (Plate 4, Figs. 20, 24, 25), IIIL-2 (Plate 7, Figs. 37, 41, 42) and a complex rearrangement in the IIL arm of at least three steps, IIL-C, or at least four steps, IIL-C.8. The less rearranged sequence, IIL-C is a feature of "Nile". "Nile" is therefore considered to be closer to "Bille" (Plate 5, Fig.26).

Distinctions between "Nile" and "Sirba" are based primarily on rearrangements in the IIL arm (Tables 6,7; Plate 6). In Nile almost all females are homozygous for IIL-C while almost all males are heterozygous for IIL-C.8. There is therefore a relation between sex and IIL-C.8 in "Nile". In contrast "Sirba" is exclusively homozygous for IIL-C.8 which shows no relation with sex. The sex determining mechanism in "Sirba" is based on a rearrangement in the IS arm, IS-3.

There appear to be "pure" populations of two kinds as shown in Tables 6 and 7, where all collections of "Nile" and "Sirba" are organized into groups in a South-North transect starting with the predominant "Nile" fauna in the southern forest region, through the Guinea Savannah to the northernly dry Sudan Savannah which is almost exclusively

a "Sirba" zone. Within the Savannah, two sections are recognized, one from which "Nile" is known to the exclusion of "Sirba" and the other in which both species are sympatric.

As shown in Tables 6 and 7, "Nile" and "Sirba" are further distinguished by shifts in the proportions of IIIL-2, IIIL-2.6, and IS-2. Generally IIIL-2 is characteristic of "Nile" while IIIL-2.6 is a feature of "Sirba"; IS-2 is virtually fixed in "Sirba" but is clearly floating in "Nile". The last two inversions are among the eight shared by these species. Both species are anthropophilic.

Elaboration on the diagnostic and shared inversions of "Nile" and "Sirba" will be considered next, starting with their sex chromosomes.

Sex Chromosomes of "Nile" and "Sirba"

The distribution of IIL-C.8 in "Nile" and "Sirba" among the sexes is shown in Tables 8a, 8b.

In "Nile" there is a clear relation between sex and IIL-C.8. No "Nile" individuals were found homozygous for IIL-C.8. Females were largely standard homozygotes for IIL-C. Males were predominantly heterozygotes for IIL-C.8.

A total of 249 individuals were homozygous for IIL-C. The majority, 217 were females; 32 were males. Conversely in a total of 199 heterozygotes, all except 7 were males.

From these data, it is clear that the X chromosome is marked by IIL-C, the Y by IIL-C.8.

What remains to be explained are the exceptions to this sex system: the 32 males homozygous for IIL-C and the 7 females heterozygotes for IIL-C.8. Three interpretations are presented for the exceptional males. It is possible that some larvae were mis-sexed, i.e., the presumed males were in fact females. Secondly, the possibility may be considered of the persistence in the population of a rare, ancestral, alternate Y chromosome, IIL-C. Finally, the exceptional Y chromosome may have arisen through crossing over in a homologous segment separating the sex locus and the sex linked inversion, analogous to the scheme proposed for IIL-18 in "Yah".

Considering next the exceptional females namely 7 heterozygotes for IIL-C.8, unless these were mis-sexed, their IIL-C.8 chromosomes probably trace back to crossover X chromosome combining the female sex locus and the inversion IIIL-C.8 which normally marks the Y chromosome. The presence of male enhancing alleles in the IIL-C.8 inversion combined with the primary female sex gene may be expected to impair fertility and thus account for the rarity of exceptional females.

As regards the distribution of IIL-C.8 in "Sirba" out of 508 individuals, there were 249 males and 259 females. Both sexes were without exception, homozygotes for IIL-C.8. It is clear that this inversion is not sex linked in "Sirba".

In contrast, in "Sirba" but not in "Nile" there is a peculiar preferential distribution of IS-3 among males and females, obviously related to sex (Tables 9a, 9b).

In "Sirba", a total of 508 specimens were sexed. Of these 153 females were standard homozygotes for IS-3 and 98 were homozygous for the inversion. Strangely enough only 8 females were heterozygotes for IS-3.

Conversely in the 249 males, the predominant class was 181 heterozygotes of IS-3, the other common class was 59 standard homozygotes of IS-3. Only 9 males were homozygous for the inversion.

In summary, females are either standard homozygotes or homozygous for IS-3. This tends to show that there are two kinds of X chromosomes : one marked by the standard arrangement and the other by the inverted sequence.

Males are either standard homozygotesor heterozygotes for IS-3. This indicates that the standard sequence is the universal Y chromosome.

If this were a panmictic population, one would expect to find females heterozygous for IS-3 through the mating of standard homozygote males and females homozygous for IS-3; or through matings

between males heterozygous for IS-3 and standard homozygote females.

The extreme rarity of such heterozygous females indicates that these matings in fact do not occur or do not produce detectable progeny. "Sirba" is therefore divided into two subsiblings, one distinguished by the standard homozygote IS-3 sequence in both males and females, the other by male heterozygosity and female homozygosity for IS-3.

These siblings effectively do not interbreed or interbreed rarely.

Occasional hybridization may explain the exceptional individuals that do not fit into either sibling; that is, the 8 S/IS-3 females and the 9 IS-3/IS-3 males.

It is possible too, as postulated for IIL-18 in "Yah" and IIL-C.8 in "Nile", that these exceptional males and females were misexed or that they carry crossover or rare, ancestral sex chromosomes.

Inversion Polymorphisms in "Nile" and "Sirba"

A total of 13 floating inversions were observed in "Nile" and "Sirba" (Tables 6, 7 and Plates 3, Figs. 16 to 18; Plate 4, Figs. 24, 25; Plate 6, Plate 7 Figs. 34 to 36.)

Where practical, inversions were tested for the Hardy-Weinberg equilibrium condition and all obeyed except sample #7 with respect to IIIL-7 ($p=0.015$) in "Nile".

Eight inversions are shared, namely, IS-2, IS-2.18, IL-2, IL-13, IIL-8.20, IIL-35, IIIL-6 and IIIL-7. In each species, 2 inversions were found restricted: IS-16 and IS-12 were observed in "Nile" while IIIL-22 and IIIL-27 were exclusive to "Sirba".

The shared rearrangements will be presented first in relation to the species and their respective geographical regions. Among the shared inversions, IIIL-6 and IS-2 will be considered in detail to show their diagnostic value. The rest of the floating inversions will be presented to demonstrate the extent of shared inversion polymorphism in "Nile" and "Sirba".

IIIL-6 This inversion floats in both species; it is based on IIIL-2 as standard. In "Nile" the heterozygous alternate, IIIL-2/IIIL-2.6 is recorded from two forest sites at Tiassale in Ivory Coast and six locations from the Savannah, i.e., one site near Badikaha in Central Ivory Coast, one further north at Leraba Bridge on the Upper Volta - Ivory Coast border, a third at Kouoro in Mali, one from Abuja and Yankpa-kainji in Nigeria and the sixth from the Mayo Salah, near Nyaoundere, W. Cameroon.

In "Nile" therefore, the distribution of this inversion appears very limited in the forest zone where a frequency of 0.004 was observed. A slight increase was observed in the Savannah where frequencies of 0.09 and 0.03 were recorded from areas where "Nile" alone was found and those in which it is sympatric with "Sirba". In general, therefore, the frequencies of the

heterozygous sequence were low in both the forest and the Savannah. The homozygous alternate, IIIL-2.6/IIIL-2.6 was not observed in "Nile".

In contrast, in "Sibra" IIIL-2.6 predominates homozygously. The incidence of the inversion is heterogeneous as shown by 2 x 3 tests. For samples # 26, and 41 the P value was 0.0003. All three zygotes of IIIL-6 were recorded from most of the collections (Table 7).. The distribution of these sequences, shows very limited records of IIIL-2 in most "Sirba" populations.

In the Sudan Savannah population where this observation was more marked, IIIL-2 was not found in 5 of the 20 locations; IIIL-2/IIIL-2.6 was not observed in 7 of the sites. IIIL-2.6/IIIL-2.6 was observed in all the locations. Even where all three zygotes were found records of the "standard" pattern IIIL-2, were generally fewer than those of either the heterozygous or homozygous rearrangements. The frequencies of IIIL-2, IIIL-2/IIIL-2.6, and IIIL-2.6 were 0.7, 0.18 and 0.75 respectively.

A similar situation was observed in "Sirba" from the Central Savannah where the frequencies are 0.3 for IIIL-2, 0.2 for IIIL-2/IIIL-2.6 and 0.5 for IIIL-2.6/IIIL-2.6.

When frequencies of IIIL-2 and IIIL-6 are compared in a South-North transect , two parallel "clines" are observed. IIIL-2 shows a frequency of 0.996 in forest "Nile", 0.91 and 0.97 in Savannah "Nile", 0.3 in Savannah "Sirba" and 0.07 in Sudan "Sirba".

These extreme shifts in the populations of IIIL-2 and IIIL-6 have led to the observation that IIIL-2 is a feature of "Nile" while IIIL-6 is characteristic of "Sirba". There are exceptions in each case; to explain these one would make the basic assumption that IIIL-2 and IIIL-6 were both present in an ancestral "Nile-Sirba" population from which emerged the "Nile" line with a predominant IIIL-2 pattern and the "Sirba" line with a predominant IIIL-6 rearrangement. On this assumption, the presence of IIIL-2 in Sirba and of IIIL-6 in Nile may be due to "carry-overs" from the ancestral population.

Another alternative is that occasional hybridization might occur between Nile and Sirba.

IS-2

This inversion is shared between "Nile" and "Sirba". All three types of zygotes were recorded in Nile. In the forest zones of Liberia and Ivory Coast, the standard arrangement was recorded from all ~~but two~~ of nine locations (Table 6).

The heterozygous inversion was found in all nine sites except one on the St. John River near Duo Town in Liberia,

The homozygous inversion was recorded from four locations in Ivory Coast: two from the Cavally River at Oua and Toulepleu and two from the Bandama River at Beoumi and Marabadiassa.

The frequencies of the standard sequence, the heterozygous sequence and the homozygous sequence were 0.48, 0.39 and 0.13 respectively. Thus the frequency of IS-2 was 0.33.

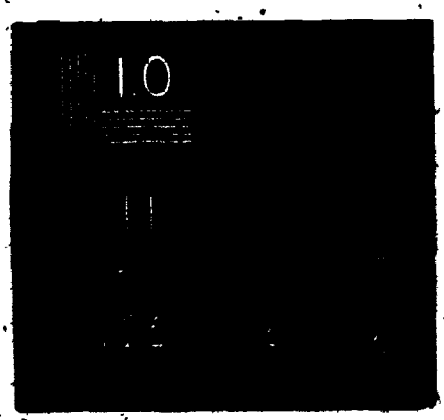
In the Savannah region where "Nile" populations exclusive of "Sirba" are known from five localities (Table 6), the heterozygous inversion was recorded from one collection on the River Leraba at the Bridge on the Upper-Volta/Ivory Coast border, one site on River Iku at Abuja, Nigeria and the third from River Mayo Boki near Nyaoundere, W. Cameroon.

The frequency of S/IS-2 was 0.31. The inversion was not observed homozygously.

In areas where "Nile" and "Sirba" are sympatric which are all in the Savannah except one forest site, the heterozygous rearrangement was observed in four of the eight locations: one from Abuja in Nigeria, the rest from the Bandama Valley at Niakaramandougou, Badikaha and Korhogo all from Ivory Coast (TAble 6). The homozygous inversion was found appreciably in most of the collections and exclusively in 3 of them. The standard pattern showed the least distribution.

2 2

OF/DE



The frequencies of the 3 zygote sequences for this zone were : 0.19 for the standard arrangement, 0.31 for the heterozygous arrangement and 0.50 for the homozygous arrangement. Thus the frequency of IS-2 was 0.81.

In "Sirba" out of 65 individuals "extracted" from 8 localities in which it is sympatric with "Nile" in the Savannah, 3 were heterozygous and 4 were standard for IS-2; the rest were homozygous for IS-2 (Table 7).

The frequency of the floating inversion was 0.92 while that for "Nile" in the same Savannah area was 0.65. In this area, differences in frequencies show more sharply when the relative frequencies of the two species are compared for the three zygote sequences of IS-2. The frequencies of "Nile" for the standard pattern, the heterozygous and homozygous, arrangements were 0.19, 0.31, and 0.50 respectively. The corresponding frequencies in "Sirba" were 0.06; 0.05 and 0.89. In "Sirba" therefore it appears IS-2 is almost fixed.

This is even more so when one considers the incidence of this inversion in "Sirba" from the Sudan Savannah or areas with a Sudan-type climate.

The distribution of IS-2 in "Sirba" from the Sudan Savannah is shown in Table 7, for the typical Sudan Savannah areas of Niger, Mali and Upper Volta and for similar localities in Northern Ghana.

In a total of 530 specimens, 524 were homozygous for IS-2; 6 were heterozygous for IS-2. The standard sequence was not observed.

The frequency of the homozygous inversion was 0.99 compared to 0.01 for the heterozygous inversion. The frequency of IS-2 was therefore 0.99 This emphasises the observation that IS-2 is almost fixed in "Sirba".

In "Nile" the incidence of IS-2 is heterogeneous as shown by 2 x3 homogeneity tests. For samples #57 and 31 the P value was 0.0015.

IIIL-7 This is a rather common floating inversion, found in "Nile" and "Sirba" (Tables 6, 7). In Nile it was recorded heterozygously from all but one of the forest locations. The inverted homozygous sequence was recorded only once from the Cavally River at Toulepleu in Ivory Coast. The frequency of the inversion in the forest zone was 0.2 .

In the Savannah it was found heterozygously in all locations except one at Abuja, Nigeria; the homozygous inversion was recorded at two sites, Badikaha and Korhogo, both in Ivory Coast.

The frequency of this inversion in Savannah areas where "clean" Nile is known was 0.16; where Nile and Sirba are sympatric the frequency was 0.5 for "Nile" and 0.45 for "Sirba".

As regards Sirba from the Sudan Savannah the heterozygous inversion was recorded from 17 of 20 sites; the homozygous inversion from 4. . The frequency of IIIL-7 in this zone was 0.28.

IL-2

This inversion shows a limited distribution in both "Nile" and "Sirba" (Table 6,7).

In the forest zone, it was recorded from the St. John River at Duo Town in Liberia, the Cestos River at the point where it meanders onto the Liberia/Ivory Coast border, from River Cavally at Toulepleu in Ivory Coast where IL-2 is also known from Beoumi, and Marabadiassa. The frequency of the heterozygous inversion was 0.08, the homozygous inversion was not found.

In the Savannah IL-2 was identified in one individual from a collection at the Leraba Bridge. A low frequency of 0.02 was observed.

Where "Nile" and "Sirba" are sympatric, in "Nile" IL-2 was found at Badikaha, Korhogo in Ivory Coast, and Abuja and Yankpa-Kainji in Nigeria; the homozygous inversion was also recorded from the same two locations in Nigeria. The frequency of IL-2 was 0.14 for Nile and 0.05 for Sirba.

In "clean" "Sirba" populations IL-2 was observed heterozygously from collections at Dienkoa Falls, Banzo and

Transilla-Meme in Upper Volta. The homozygous inversion was not found and the frequency of the heterozygous inversion was 0.05.

An included inversion, IL-2.13 was recorded from Yankpa-Kainji, NIgeria and Kouoro in Mali. This inversion is readily recognized as a complex rearrangement involving IL-1 IL-3 and IL-13 on one constituent and IL-1, IL-3 and IL-2 on the other; the resulting configuration being IL-3.13+1/IL-3.2.1. The frequency of IL-2.13 was 0.04 for "Nile" and 0.05 for "Sirba".

IL-13 This inversion was presented above included in IL-2. It has been observed independent of IL-2 in "Nile" and "Sirba" (Table 6,7). In "Nile" it was observed heterozygously at Niakaramandougou in Ivory Coast; the homozygous inversion is known from Kouoro in Mali. A frequency of 0.01 was observed.

In "Sirba" the inversion was observed heterozygously from a single collection at Kwaregenou on the River Sirba in Niger. Here too the frequency is very low, 0.002.

The occurrence of IL-13 independent of IL-2 is as expected because as an included inversion, IL-13 is separable from IL-2 by crossing over.

IS-2.18 This too is a rare included inversion (Table 6,7), and was observed heterozygously only. Except for one record

in "Sirba" from Nasia near Bolgatanga in Northern Ghana, all other 8 records of its incidence are in "Nile" (Table 6).

In the forest zone, this inversion was recorded from the Cestos River between Liberia and Ivory Coast, the Cavally River at Touiepleu and the Bandama River at Marabadiassa and Beoumi. A frequency of 0.04 was observed.

In 5 localities in the Savannah where "Nile" is known, one record of the inversion was observed at Abuja in Nigeria. A frequency of 0.04 identical to that in the forest zone was observed. A slightly higher frequency of 0.06 was found in "Nile" in Savannah areas where this species is sympatric with "Sirba".

IIL-20 This included inversion is also shared by "Nile" and "Sirba". Since it is included in IIL-C.8, the standard sequence was considered to be IIL-c.8/IIL-C.8, the heterozygous as IIL-C.8/IIL-C.8.20 and the homozygous as IIL-C.8.20/IIL-C.8.20. The homozygous inversion was not found and frequencies were recorded for the heterozygous inversion only.

This inversion shows a wide distribution (Table 6,7) though quantitatively it is scanty. It is known from "Nile" in the forest and Savannah zones and shows frequencies of 0.03 and 0.20 respectively. Where "Nile" and "Sirba" overlap in

the Savannah, the frequencies are 0.06 for "Nile" and 0.26 for "Sirba" in the Sudan a frequency of 0.15 was recorded for "Sirba".

IIL-35 This inversion showed a very limited distribution. Only the heterozygous inversion was observed in both "Nile" and "Sirba". In "Nile" it is known from one forest location on the Cestos River, near Liberia/Ivory Coast border and one from Beoumi west of the Cestos. The frequency was 0.004.

It was not found in "Nile" from the Guinea Savannah. The only record in "Sirba" came from a collection from Korhogo in Ivory Coast. The frequency was 0.03.

In Sudan "Sirba" it was observed from two locations at De Dougou-Nouna, one from De Dougou-Tougan in Upper Volta and a fourth from Akosombo Dam in Ghana. A frequency of 0.01 was observed.

IS-16 This inversion is restricted to "Nile" but is rare and was observed heterozygously only. It is known from one forest site on the Cayally River at Toulepleu and shows a frequency of 0.004. In the Savannah it was recorded from one collection at Niakaramandougou. A frequency of 0.005 was observed.

IS-12 This inversion is exclusive to Nile but is rare. It is known heterozygously from one collection on R.Mayo Salah, near Ngaoundere, Cameroon. A frequency of 0.07 was observed.

IIL-36 This inversion was found in "Nile" only, it is rare and is known heterozygously only. It was recorded from Abuja in Nigeria and the Leraba Bridge. A frequency of 0.09 was observed.

It is also known from Badikaha where "Nile" and "Sirba" are sympatric. The frequency was 0.01.

IIIL-22 This inversion is restricted to "Sirba". It is rare and was observed heterozygously from a collection at Yankpa-Kainji in Nigeria. It showed a frequency of 0.02.

In Sudan Savannah "Sirba" it is known from Samandeni and De Dougou-Nouna. A frequency of 0.004 was observed.

IIIL-27 This inversion is restricted to "Sirba" but is rare and only its heterozygous rearrangement was observed at De Dougou-Tougan in Upper Volta and Nangodi in Northern Ghana. A frequency of 0.004 was observed.

The value of these restrictive inversions in "Nile" and "Sirba" in species delimitation is greatly reduced by their rarity. If they had been observed frequently enough, they might have been used as further evidence of distinction between "Nile" and "Sirba" as shown by their different sex determining chromosomes, shifts in the proportions of IIIL-2 and IIIL-6 and the observation that IS-2 floats in "Nile" whereas it is almost fixed in "Sirba".

Distribution of "Nile and Sirba"

"Nile" and "Sirba" are known from many localities in the forest and Savannah zones of West Africa (Map 3).

"Nile" has been identified from collections in the forest, wood-land Savannah and grass-land Savannah regions of Cameroon, Nigeria, Liberia, Ivory Coast, Ghana, Mali and Niger. This species predominates in the forest region, its records diminishing inland as the forest approaches the southern Guinea Savannah where records of "Sirba" though still less predominant, are quite appreciable.

The distribution of "Sirba" appears to be a direct reversal of "Nile's". "Sirba" is predominant in the almost arid Sudan Savannah.

All collections from Niger and most samples from Mali, Upper Volta and Ghana were exclusively "Sirba". Further South, "Sirba" decreases quantitatively and almost progressively, through the Savannah belt to the forest zone where scanty records of this species are obvious.

In Dahomey, "Sirba" is known from the Mono River in the "Dahomey Gap" which is an intrusion of Savannah into the Coastal forest zone. In this area, "Sirba" is sympatric with "Soubre".

In general therefore, the distribution of "Nile" and "Sirba" appears to coincide with the two major geographic regions, forest and Savannah; in both regions, I experienced ferocious biting by S. damnosum and saw various effects of onchocerciasis in known "Nile" and "Sirba" localities as also reported by Garms (1973), Le Berre et al (1964), Davies (1963, 1968) and Duke et al (1966). It therefore appears that both species are implicated in the transmission of onchocerciasis.

"Dieguera" Sibling

This species differs from "Bille" by IIL-C.8, IL-12, IS-2 and IIIL-2. Only one floating inversion IIIL-28 was recorded and this is restricted to "Dieguera".

Of the interspecific inversions, IIL-C.8 is similarly fixed in "Sirba", IS-2 floats in "Nile" and Sirba while IL-12 floats in "Yah". In Dieguera, however, homozygosity which indicates the fixation of rearrangements, was observed in all individuals for IL-12 and IS-2. No sex chromosomes were observed in this species.

"Dieguera" is known from a single collection from River Bafing at Dieguera in Mali where it is sympatric with "Sirba". There is no information on the biology or biting habits of this species.

Since available information on "Dieguera" is based on only five individuals, further investigation is required to determine the distribution of the species.

TABLE - 6 : Inversion Polymorphisms in "Nile"

TABLE 6

Inversion Polymorphism in 'Nile'

1 of

TABLE 6 (Cont'd)

		Forest										G. Savannah						(Extract		(Extracted
Sample # *		59	60	57	50	1	5	6	7	8	Total	31	33	35	36	14	Total	17	18	18
Total larvae		19	7	62	27	9	1	3	82	11	221	22	1	2	10	10	45	2	2	2
	** C/C	10	4	34	15	5	1	2	45	6	122	22	-	2	6	2	32	1		-
IIL-C	C/C.8	9	3	28	12	4	-	1	37	5	99	-	1	-	4	8	13	1	2	2
	C.8/C.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	19	7	58	23	1	1	2	53	5	169	14	1	2	9	6	32	1		1
IS-3	S/3	-	-	4	4	1	-	1	29	6	45	8	-	-	1	4	13	-		1
	3/3	-	-	-	-	7	-	-	-	-	7	-	-	-	-	-	-	1		-
	2/2	19	7	62	27	8	1	3	82	11	220	21	1	2	9	8	41	1		1
IIIL-6	2/2.6	-	-	-	-	1	-	-	-	-	1	1	-	-	1	2	4	1		1
	2.6/2.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	11	7	51	16	8	-	1	12	-	105	12	1	1	10	4	28	-		-
IS-2	S/2	8	-	9	8	1	1	2	53	5	88	10	-	1	-	6	17	-		-
	2/2	-	-	2	3	-	-	-	17	6	28	-	-	-	-	-	-	2		2
	S/2.18	-	-	1	1	-	-	-	6	2	10	2	-	-	-	-	2	-		-
IS	S/16	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-		-
	S/12	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	3	-		-
	S/S	19	5	58	26	9	1	3	75	7	203	22	1	2	10	9	44	2		2
IL-2	S/2	-	2	4	1	-	-	-	7	4	18	-	-	-	-	1	1	-		-
	2/2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	2/13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	19	7	62	27	9	1	3	82	11	221	22	1	2	10	10	45	2		2
IL-13	S/13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	13/13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	19	7	62	26	8	1	3	81	10	217	22	-	2	10	10	44	2		2
IIL-20	S/8.20	-	-	-	1	1	-	-	1	1	4	-	1	-	-	-	1	-		-
	8/8.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	-	-	-	-	-	-	-	-	-	-	22	1	2	10	10	45	2		2
IIL-35	S/35	-	-	3	-	-	-	-	1	-	-	-	-	-	-	-	-	-		-
	35/35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	-	-	-	-	-	-	-	-	-	-	19	1	2	10	9	41	2		2
IIL-36	S/36	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1	4	-		-
	36/36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-
	S/S	11	5	44	13	5	1	2	48	6	135	22	-	1	8	7	38	2		2
IIIL-7	S/7	8	2	18	13	4	-	1	34	5	85	-	1	1	2	3	7	-		-
	7/7	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-		-
	S/S																			
IIIL-22	S/22																			
	22/22																			
	S/S																			
IIIL-27	S/27																			
	27/27																			

* For details of locations refer to Table 10

** In each of zygote sequences the first is considered standard

Where entry is not made standard is assumed

TABLE 6 (Cont'd)

Savannah
(Extracted from mixed populations of 'Nile' and 'Sirba')

18	20	24	25	26	27	Total
2	1	1	1	2	1	10
-	1	-	-	-	-	2
2	-	1	1	2	1	8
-	-	-	-	-	-	-
1	1	1	1	2	1	8
1	-	-	-	-	-	1
-	-	-	-	-	-	1
1	1	1	1	2	1	8
1	-	-	-	-	-	2
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	1	-	1
2	1	1	1	1	1	9
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
2	1	1	1	2	1	10
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	-	-	-	-	-
2	1	1	1	2	1	10
-	-	-	-	-	-	-
-	-	-	-	-	-	-
2	1	1	1	2	1	10
-	-	-	-	-	-	-
-	-	-	-	-	-	-
2	1	1	1	2	1	10
-	-	-	-	-	-	-
-	-	-	-	-	-	-
2	1	1	1	2	1	10
-	-	-	-	-	-	-
-	-	-	-	-	-	-
2	1	1	1	2	1	10
-	-	-	-	-	-	-
-	-	-	-	-	-	-

(Extracted from mixed populations of 'Nile' and 'Sirba') : Savannah

9	10	11	12	32	34	38	30	Total
48	67	38	5	7	20	25	7	217
29	49	19	5	3	5	10	3	123
19	18	19	-	4	15	15	4	94
-	-	-	-	-	-	-	-	-
27	45	25	4	4	8	13	5	131
21	21	11	1	2	12	9	1	78
-	1	2	-	1	-	3	1	8
48	66	38	4	7	17	23	7	210
-	1	-	1	-	3	2	-	7
-	-	-	-	-	-	-	-	-
4	21	14	-	2	-	-	-	41
15	34	18	-	1	-	-	-	68
29	12	6	5	4	20	25	7	108
3	5	6						14
1	-	-	-	-	-	-	-	1
-	-	-	-	-	-	-	-	-
48	59	32	5	1	7	19	7	178
-	8	6	-	3	7	-	-	24
-	-	-	-	3	4	-	-	7
-	-	-	-	-	2	6	-	8
48	66	38	5	7	20	24	7	215
-	1	-	-	-	-	-	-	1
-	-	-	-	-	-	1	-	1
48	66	35	5	6	20	20	5	205
-	1	3	-	1	-	5	2	12
-	-	-	-	-	-	-	-	-
48	67	38	5	7	20	25	7	217
-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-
48	64	38	5	7	20	25	7	214
-	3	-	-	-	-	-	-	3
-	-	-	-	-	-	-	-	-
22	28	17	4	5	12	22	6	116
26	38	19	1	2	8	3	1	98
-	1	2	-	-	-	-	-	3

TABLE - 7 : Inversion Polymorphisms in "Sirba"

TABLE 7

Inversion Polymorphism in 'Sirba'

1 of

(Extracted from mixed populations of 'Nile and 'Sirba') Savannah

Sample # *		9	10	11	12	32	34	38	30	Total
Total Larvae		4	21	12	9	2	6	8	3	65
IIL-C	** C/C	-	-	-	-	-	-	-	-	-
	C/C.8	-	-	-	-	-	-	-	-	-
	C.8/C.8	4	21	12	9	2	6	8	3	65
IS-3	S/S	-	10	8	5	1	3	4	1	32
	S/3	1	5	2	3	1	3	3	2	20
	3/3	3	6	2	1	-	-	1	-	13
IIIL-6		1	5	2	2	2	-	7	3	22
	2/2.6	-	6	4	2	-	-	1	-	13
	2.6/2.6	3	10	6	5	-	6	-	-	30
IS-2	S/S	-	1	3	-	-	-	-	-	4
	S/2	-	2	1						3
	2/2	4	18	8	9	2	6	8	3	58
IS	S/2.18	-	-	-	-	-	-	-	-	-
	S/16	-	-	-	-	-	-	-	-	-
	S/12	-	-	-	-	-	-	-	-	-
IL-2	S/S	4	19	12	9	1	5	5	3	58
	S/2	-	1	-	-	1	1	-	-	3
	2/2	-	-	-	-	-	-	-	-	-
	2/13	-	-	-	-	-	-	3	-	3
IL-13	S/S	4	20	12	9	2	6	8	3	64
	S/13	-	1	-	-	-	-	-	-	1
	13/13	-	-	-	-	-	-	-	-	-
IIL-20	S/S	4	12	10	8	-	6	8	-	48
	S/8.20	-	-	-	-	-	-	-	-	-
	8/8.20	-	9	2	1	2	-	-	3	17
IIL-35	S/S	4	21	12	7	2	6	8	3	63
	S/35	-	-	-	2	-	-	-	-	2
	35/35	-	-	-	-	-	-	-	-	-
IIL-36	S/S	4	21	12	9	2	6	8	3	65
	S/36	-	-	-	-	-	-	-	-	-
	36/36	-	-	-	-	-	-	-	-	-
IIIL-7	S/S	3	10	4	4	2	2	8	3	36
	S/7	1	11	6	4	-	4	-	-	26
	7/7	-	-	2	1	-	-	-	-	3
IIIL-22	S/S	4	21	12	9	2	5	8	3	64
	S/22	-	-	-	-	-	1	-	-	1
	22/22	-	-	-	-	-	-	-	-	-
IIIL-27	S/S									
	S/27									
	27/27									

TABLE 7 (Cont'd)

Forest							S. Savannah
50	1	37	39	15	17	18	20
1	1	16	7	8	3	20	26
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
1	1	16	7	8	3	20	26
-	-	16	3	7	-	4	5
-	-	-	4	1	2	9	15
1	1	-	-	-	1	7	6
-	1	-	1	2	-	-	1
-	-	-	-	1	-	5	5
1	-	16	6	5	30	15	20
-	-	-	-	-	-	-	-
-	-	-	-	1	-	1	-
1	1	16	7	7	3	19	26
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
1	1	16	7	8	2	19	26
-	-	-	-	-	1	1	-
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
1	1	15	7	8	3	20	26
-	-	-	-	-	-	-	-
-	-	1	-	-	-	-	-
-	-	16	7	7	2	17	15
-	-	-	-	-	-	-	-
1	1	-	-	1	1	3	11
1	1	16	7	8	3	20	26
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
1	1	16	7	8	3	20	26
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-
-	1	16	6	4	2	14	13
1	-	-	1	4	1	6	12
-	-	-	-	-	-	-	1
1	1	16	7	8	3	20	26
-	-	-	1	-	-	-	-
-	-	-	-	-	-	-	-
1	1	16	7	8	3	20	26
-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-

* For details of locations refer to Table 10

** In each of the zygote sequences, the first is considered standard where entry is not made standard is assumed

'd)

2 of 2

S. Savannah

39	15	17	18	20	21	22	23	24	25	26	27	28	29	13	40	41	42	43	Total
7	8	3	20	26	10	20	5	87	28	28	102	72	35	11	9	26	4	11	530
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	8	3	20	26	10	20	5	87	28	28	102	72	35	11	9	26	4	11	530
3	7	-	4	5	5	11	2	18	6	9	66	25	8	1	6	26	3	9	230
4	1	2	9	15	3	9	2	36	4	16	24	33	15	6	1	-	-	1	181
-	-	1	7	6	2	-	1	33	18	3	12	14	12	4	2	-	1	1	119
1	2	-	-	1	5	2	2	-	-	4	3	1	-	-	-	-	2	7	31
-	1	-	5	5	4	5	-	13	13	7	18	11	6	4	-	-	-	-	93
6	5	30	15	20	1	13	3	74	15	17	81	60	29	7	9	26	2	4	406
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	1	-	1	-	-	-	-	-	-	2	-	2	-	-	-	-	-	-	6
7	7	3	19	26	10	20	5	87	28	26	102	70	35	11	9	26	4	11	524
-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	8	2	19	26	9	20	5	87	28	28	102	72	35	11	9	26	4	6	522
-	-	1	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	3	6
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	8	3	20	26	10	20	5	87	28	28	102	72	35	11	9	26	4	11	530
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	7	2	17	15	8	14	5	74	21	25	92	58	31	7	9	26	4	11	449
-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1
-	1	1	3	11	2	6	-	13	6	3	10	14	4	4	-	-	-	-	80
7	8	3	20	26	10	20	4	86	27	28	102	70	35	11	9	26	4	11	525
-	-	-	-	-	-	-	1	1	1	-	-	2	-	-	-	-	-	-	5
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	8	3	20	26	10	20	5	87	28	28	102	72	35	11	9	26	4	11	530
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	4	2	14	13	7	7	4	68	19	21	71	47	24	5	9	25	3	11	377
1	4	1	6	12	3	11	1	19	9	7	30	24	11	6	-	1	1	-	148
-	-	-	-	1	-	2	-	-	-	-	1	1	-	-	-	-	-	-	5
8	8	3	20	26	10	19	5	86	28	28	102	72	35	11	9	26	4	11	528
-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	8	3	20	26	10	20	5	87	27	28	102	71	35	11	9	26	4	11	528
-	-	-	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-	-	2
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 8 a

The Relation between Sex and IIL-C.8 in 'Nile'

'NILE' Sample #	Total larvae	C/C ♂	C/C ♀	C/C.8 ♂	C/C.8 ♀	C.8/C.8 ♂	C.8/C.8 ♀	Sex undetermined C/C	Sex undetermined C/C.8	Sex undetermined C.8/C.
59	19	-	10	9	-	-	-	-	-	-
60	7	-	4	3	-	-	-	-	-	-
57	62	5	26	26	1	-	-	3	1	-
50	27	2	11	9	-	-	-	2	3	-
1	9	-	4	4	-	-	-	1	-	-
5	1	-	1	-	-	-	-	-	-	-
6	3	-	2	1	-	-	-	-	-	-
7	82	4	31	34	-	-	-	10	3	-
8	11.	1	5	5	-	-	-	-	-	-
Total	221	12	94	91	1	-	-	16	7	-
31	22	11	9	-	-	-	-	2	-	-
33	1	-	-	1	-	-	-	-	-	-
35	2	-	-	-	-	-	-	2	-	-
36	10	-	6	1	2	-	-	-	1	-
14	10	-	2	8	-	-	-	-	-	-
Total	45	11	17	10	2	-	-	4	1	-
17	2	-	1	1	-	-	-	-	-	-
18	2	-	-	2	-	-	-	-	-	-
20	1	-	1	-	-	-	-	-	-	-
24	1	-	-	1	-	-	-	-	-	-
25	1	-	-	1	-	-	-	-	-	-
26	2	-	-	2	-	-	-	-	-	-
27	1	-	-	1	-	-	-	-	-	-
Total	10	-	2	8	-	-	-	-	-	-
9	48	2	26	15	1	-	-	1	3	-
10	67	2	44	15	3	-	-	3	-	-
11	38	-	19	17	-	-	-	-	2	-
12	5	1	1	-	-	-	-	3	-	-
32	7	-	3	3	-	-	-	-	1	-
34	20	1	4	15	-	-	-	-	-	-
38	25	3	6	14	-	-	-	1	1	-
30	7	-	1	4	-	-	-	2	-	-
Total	217	9	104	83	4	-	-	10	7	-
Grand total	493	32	217	192	7	-	-	30	15	-

TABLE 8b

The Relation between Sex and IIL-C.8 in 'Sirba'

'SIRBA' Sample#	Total larvae	C/C ♂	C/C ♀	C/C.8 ♂	C/C.8 ♀	C.8/C.8 ♂	C.8/C.8 ♀	Sex undetermined C/C	Sex undetermined C/C.8	Sex undetermined C.8/C.
9	4	-	-	-	-	1	3	-	-	-
10	21	-	-	-	-	8	13	-	-	-
11	12	-	-	-	-	2	8	-	-	2
12	9	-	-	-	-	4	3	-	-	2
32	2	-	-	-	-	2	-	-	-	-
34	6	-	-	-	-	6	-	-	-	-
38	8	-	-	-	-	4	3	-	-	1
30	3	-	-	-	-	3	-	-	-	-
Total	65	-	-	-	-	30	30	-	-	5
50	1	-	-	-	-	-	1	-	-	-
1	1	-	-	-	-	-	1	-	-	-
37	16	-	-	-	-	2	11	-	-	3
39	7	-	-	-	-	3	3	-	-	1
15	8	-	-	-	-	3	-	-	-	5
17	3	-	-	-	-	1	2	-	-	-
18	20	-	-	-	-	8	5	-	-	7
20	26	-	-	-	-	16	6	-	-	4
21	10	-	-	-	-	2	7	-	-	1
22	20	-	-	-	-	3	7	-	-	10
23	5	-	-	-	-	2	-	-	-	3
24	87	-	-	-	-	41	37	-	-	9
25	28	-	-	-	-	5	17	-	-	6
26	28	-	-	-	-	18	10	-	-	-
27	102	-	-	-	-	38	61	-	-	3
28	72	-	-	-	-	37	30	-	-	5
29	35	-	-	-	-	22	10	-	-	3
13	11	-	-	-	-	4	2	-	-	5
40	9	-	-	-	-	3	3	-	-	3
41	26	-	-	-	-	8	6	-	-	12
42	4	-	-	-	-	-	4	-	-	-
43	11	-	-	-	-	3	6	-	-	2
Total	530	-	-	-	-	219	229	-	-	82
Grand total	595	-	-	-	-	249	259	-	-	87

TABLE 9a

The Relation Between Sex and IS-3 in 'Nile'

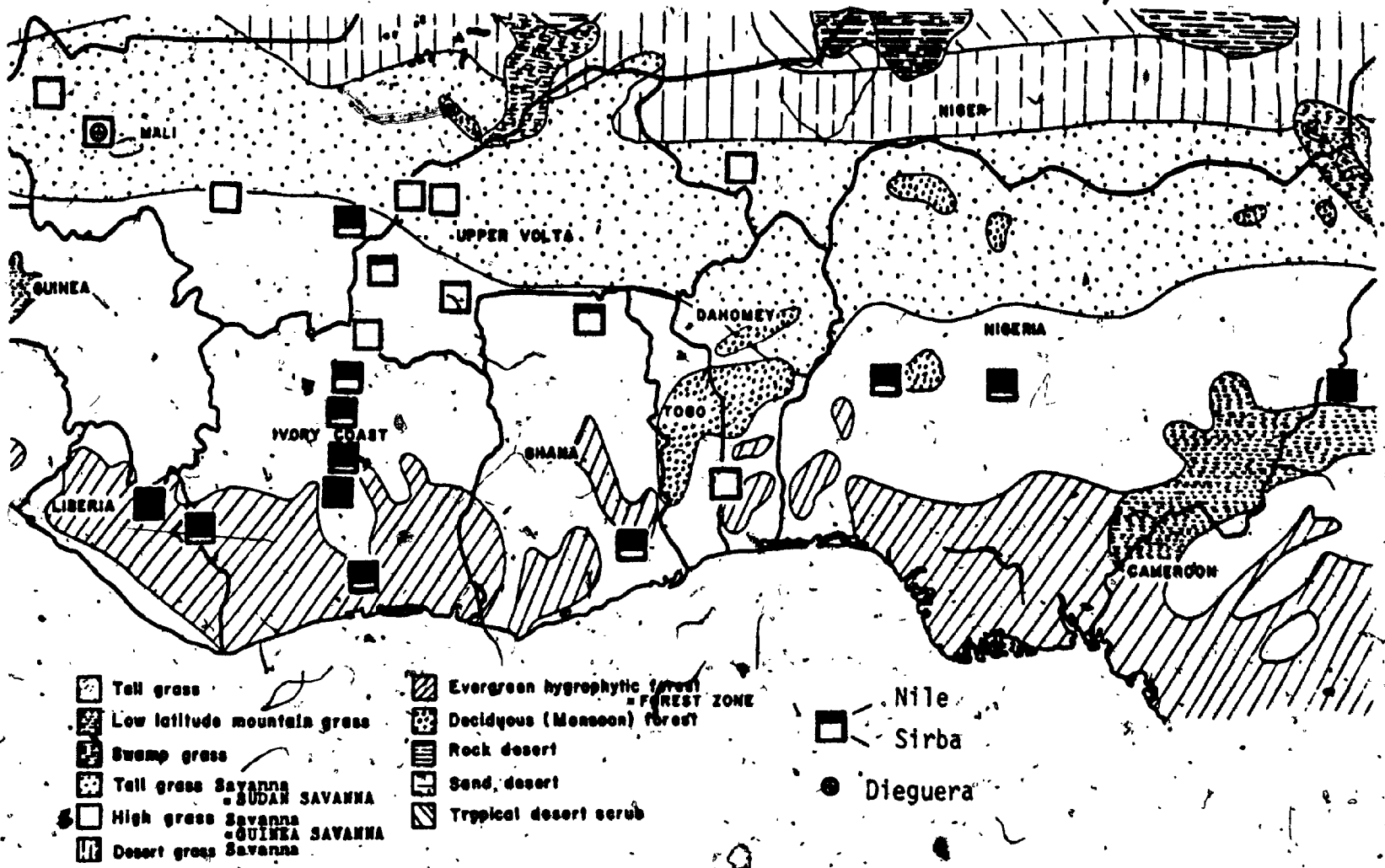
Sample #	'NILE' Total larvae	S/S ♂	S/S ♀	S/3 ♂	S/3 ♀	3/3 ♂	3/3 ♀	Sex undetermined S/S	Sex undetermined S/3	Sex undetermined 3/3
59	19	9	10	-	-	-	-			
60	7	3	4	-	-	-	-			
57	62	30	25	1	2	-	-	3	1	-
50	27	9	11	2	-	-	-	3	2	-
1	9	-	1	1	-	3	3	-	-	1
5	1	-	1	-	-	-	-			
6	3	1	1	-	1	-	-			
7	82	20	23	18	8	-	-	10	3	-
8	11	3	2	3	3	-	-			
Total	221	75	78	25	14	3	3	16	6	1
31	22	6	7	5	2	-	-	1	1	-
33	1	1	-	-	-	-	-	-	-	-
35	2	-	-	-	-	-	-	2	-	-
36	10	1	7	-	1	-	-	1	-	-
14	10	4	2	4	-	-	-			
Total	45	12	16	9	3	-	-	4	1	-
17	2	-	1	-	1	-	-			
18	2	1	-	1	-	-	-	-	-	-
20	1	-	1	-	-	-	-	-	-	-
24	1	1	-	-	-	-	-	-	-	-
25	1	1	-	-	-	-	-	-	-	-
26	2	2	-	-	-	-	-	-	-	-
27	1	1	-	-	-	-	-	-	-	-
Total	10	6	2	1	1	-	-	-	-	-
9	48	12	11	5	16	-	-	4		
10	67	14	28	3	18	-	1	3		
11	38	15	10	1	8	1	1	-	2	-
12	5	-	1	1	-	-	-	3		
32	7	2	2	1	-	-	1	-	1	-
34	20	5	3	11	1	-	-	-	-	-
38	25	8	3	9	-	-	3	2		
30	7	3	1	1	-	-	-	1	-	1
Total	217	59	59	32	43	1	6	13	3	1
Gran Total	493	152	155	67	61	4	9	33	10	2

TABLE 9b

The Relation Between Sex and IS-3 in 'Sirba'

'SIRBA' Sample#	Total larvae	S/S ♂	S/S ♀	S/3 ♂	S/3 ♀	3/3 ♂	3/3 ♀	Sex undetermined S/S	Sex undetermined S/3	Sex undetermined 3/3
9	4	-	-	1	-	-	3			
10	21	3	7	5	-	-	6			
11	12	-	7	2	-	-	1	1		1
12	9	2	1	2	1	-	1	2		
32	2	1	-	1	-	-	-			
34	6	3	-	3	-	-	-			
38	8	1	2	3	-	-	1	1		
30	3	1	-	2	-	-	-	-		
Total	65	11	17	19	1	-	12	4	-	1
50	1	-	-	-	-	-	1			
1	1	-	-	-	-	-	1			
37	16	2	11	-	-	-	-	3		
39	7	-	2	3	1	-	-	1		
15	8	2	-	1	-	-	-	5		
17	3	-	-	1	-	-	2			
18	20	1	-	7	-	-	5	3	2	2
20	26	1	2	14	-	1	4	2	1	1
21	10	-	5	2	1	-	1	-	-	1
22	20	-	5	3	2	-	-	6	4	-
23	5	-	-	2	-	-	-	2	-	1
24	87	4	12	35	1	2	24	2	-	7
25	28	-	3	4	-	1	14	3	-	3
26	28	1	8	16	-	1	2			
27	102	15	49	23	-	-	12	2	1	-
28	72	4	19	32	-	1	11	2	1	2
29	35	4	4	15	-	3	6	-	-	3
13	11	-	1	4	1	-	-	-	1	4
40	9	3	2	-	-	-	1	1	1	1
41	26	8	6	-	-	-	-	12		
42	4	-	3	-	-	-	1			
43	11	3	4	-	1	-	1	2		
Total	530	48	136	162	7	9	86	46	11	25
Grand Total	595	59	153	181	8	9	98	50	11	26

MAP - 3 : The Distribution of "Nile", "Sirba" and "Dieguera"


MALI
NIGER
UPPER VOLTA
GUINEA
DAHOMEY
NIGERIA
IVORY COAST
GHANA
TOGO
LIBERIA
CAMEROON
Tall grass
Low latitude mountain grass
Swamp grass
Tall grass Savanna
High grass Savanna
Desert grass Savanna
= SUDAN SAVANNA
= GUINEA SAVANNA
Evergreen hygrophytic forest
Deciduous (Monsoon) forest
= FOREST ZONE
Rock desert
Sand, desert
Tropical desert scrub
Nile
Sirba
Dieguera

CHAPTER 6

DISCUSSION

Detailed analysis of the banding pattern of polytene chromosome in many instances has led to the discovery of discrete sibling species within previously supposed unitary species as shown by Dunbar (1966) in Eusimulium congareenarum Dunbar (1969) in the "Sanje" subgroup of S. damnosum, Landau (1962) in S. tuberosum, Madahar (1968) in "seven taxa in the subgenus Stegopterna", Newman (1972) in Prosimulium onychodactylum and Bedo (1973) in S. pictipes. Similar findings have been published in Drosophila by Dobzhansky (1972), in Chironomids as summarised by Wulker, Sublette and Martin (1968), in the Anopheles gambiae complex by Colluzzi and Sabatini (1967, 1968, 1969), in Anopheles faraut by Bryan and Coluzzi (1971), and in the Anopheles maculipennis complex by Kitzmiller, Frizzi and Baker (1967).

From the biological considerations as shown by Le Berre et al (1964), Lewis and Duke (1966), Duke et al (1966) and Garms (1973) and evidence from chromosome studies, S. damnosum is a species complex.

The present studies indicate that in West Africa, the S. damnosum complex includes seven sibling species. Their principal cytological characters are indicated in Plates 2 and 8.

This species complex comprises three main lineages, namely, "Bille-Yah", "Bandama-Soubre" and "Nile-Sirba-Dieguera".

Specific chromosome arrangements characterize each species. All rearrangements except IIL-C could be reduced to the same standard map. Members of each lineage group share a rare heterozygous inclusion in the IIIL arm (Plate 7, Fig.38).

Evidence that populations sharing the same gene sequence constitute distinct species includes the existence of sympatric populations in which the integrity of each species is maintained, i.e. specific inversion differences are conserved, discrete sex chromosome mechanisms and segregated inversion polymorphisms remain distinct. These criteria will be discussed in relation to the species of the S. damnosum complex.

"Bille" and "Yah" show no specific inversion differences. But "Yah" is characterized by ectopically paired heavy centromeres which permit recognition of the species at a glance through the microscope. In addition "Bille" and "Yah" have separate sex determining chromosomes. Sex determination in "Bille" is based on C_I which is heterozygous in about half of the males, homozygous in the rest and in all females. These observations indicate that in "Bille" there are two types of Y chromosomes and one kind of X chromosome.

In "Yah" on the other hand sex determination is based on IIL-18 for which about 46% of the males are heterozygous; the rest and almost all females are homozygous for IIL-18. There is evidence therefore in "Yah" of two kinds of Y chromosomes and one kind of X chromosome .

Of the eight intraspecific rearrangements of the "Bille-Yah" pair, IS-2, IS-11, IL-15, IIIS-5 and IIIL-16 are exclusive to "Bille" while IL-12 and IIL-40 are restricted to "Yah". Only IIS-6 is shared.

"Bille" is known from Cameroon and Upper Volta (Map1) where it has been recorded from relatively large rivers. "Typical forest transmission" of onchoceriasis has been reported by Duke (1971, personal communication) from Cameroon and by Philippon (1971, 1973, personal communication) from Upper Volta in known "Bille" localities. This species is therefore considered a vector of the disease.

"Yah" is known from small tributaries in Liberia and Ivory Coast. Even in Liberia where some rivers and creeks are a few miles apart, "Yah" has never been recorded from the rivers.

Garms considers "Yah" a possible transmitter of the disease. In Ivory Coast, Phillippon (1971, 1973, personal communication) has observed "zoophilic females" in some known "Yah" localities.

"Soubre" is recognized by IIL-6 while "Bandama" is characterized by IIL-6.7. They are further distinguished by restricted inversion polymorphism. "Soubre" alone has IIIL-24, IIIL-25 and IIIL-26 while IIIL-5 and IIIL-23 are exclusive to "Bandama". Also known only in "Bandama" populations are IS-20, IS-21, IL-18 and IIIL-30. These showed very low frequencies and are therefore of little value in

diagnosis. Of value in this regard is the distribution, sympatry and allopatry, exhibited by this pair.

The geographic distribution of "Bandama" and "Soubre" in an east-west transect conveys this picture in the forest zone: isolated and sympatric populations of "Bandama" and "Soubre" are known on the Bandama Valley in Ivory Coast. On the Sassandra west of the Valley, only sympatric populations of the species have been observed. On the Cavally which flows on the Ivory Coast/Liberia border, "Bandama" alone is known. West of the Cavally is the Liberian Cestos which proved to be an exclusive "Soubre" habitat. Parallel to the Cestos and to the West is a succession of four rivers : St. John, St. Paul, Lofa and Mano. Records of "Bandama" alone are known from these rivers. There is therefore indication that in the east, for example on the "Bandama" and Sassandra Valleys in Ivory Coast, "Bandama" and "Soubre" are appreciably sympatric, in the west, in Liberia, their distribtuion is segregated.

Bio-ecological differences between "Bandama" and "Soubre" are reported by Garms (1973), who notes that "Soubre" larvae settle in masses on rocks in sharp contrast to those of other species including "Bandama" which are usually found attached to vegetation; and that in localities where "Soubre" predominates, man has not been "conspicuously approached" by black flies. It therefore appears that in Liberia, "Bandama" is a transmitter of the disease, "Soubre" is not. In Ivory Coast, Phillipon (1971, 1973 personal communication) suggests that both species are probably vectors of river blindness.

The principal features that distinguish "Nile" from "Sirba" are summarized in Plate 9. "Nile" is defined by the complex rearrangement IIL-C, a preponderance of IIIL-2 and XY sex chromosomes based on IIL-C.8.

"Sirba" is characterized by IIL-C.8 which in this sibling shows no differential relation to sex, IIIL-2.6 as well as IS-2 are predominantly homozygous. There are two types of X chromosomes and one of Y. Both are based on IS-3. There are strong indications that "Sirba" is in turn divided into two subsiblings one characterized by female homozygotes of IS-3 the other by females homozygous for the inversion IS-3.

Both "Nile" and "Sirba" are known in numerous areas where onochocerciasis is endemic and are therefore considered to be the worst vectors of the diseases.

The large ranges of "Nile" and "Sirba in West Africa extend from the humid coastal forest belt where "Nile" predominates, through the Guinea Savannah where the species overlap, to the almost arid Sudan Savannah in which "Sirba" is known almost to the exclusion of "Nile".

In "Yah" for instance, the standard constituent and IIL-18 serve as alternate Y chromosomes, whereas virtually all X chromosomes are IIL-18. In contrast, in "Bille" IIL-18 is rare and apparently autosomal.

Accepting the generalization of Rothfels (1956) that an inversion per se is not a sex determiner but serves to enhance a primary mechanism, the sex locus may be located outside the inversion but closely linked to it.

In terms of the phylogeny shown in Plate 9, it may be postulated that the first step in the evolution of sex chromosomes of "Yah" was the "invention" of IIL-18. This persists as a rare autosomal inversion polymorphism in "Bille". In "Yah", the primary sex locus was - or became - located in IIL. Through crossing over between the sex locus and IIL-18 the inversion was transferred to both genetic X and Y chromosomes. Further evolution of the X chromosomes involved coadaptation of modifiers in the inversion with the primary locus and resulted in the complete or almost complete elimination of the standard sequence X chromosome. As far as the Y chromosome is concerned both the original and the IIL-18 type persisted since presumably both are capable of giving approximately equally functional males.

The exceptional X chromosomes (standard) may be relics of the ancestral X that have not yet been completely eliminated or they may be generated at a low rate by crossing over in the homozygous segment between the sex locus and the IIL-18 inversion in S/IIL-18 males.

The same situation applies to sex determination in "Bille" where a novel Y chromosome (Y_1) modified in the centromere region exists side by side with the original Y (Y_0) that is indistinguishable from the standard pattern and the X chromosome.

A similar but more complex situation is found in "Nile" and "Sirba". IIL-C.8 which is a sex chromosome in "Nile" is autosomal in "Sirba". Conversely, IS-3 on which the sex mechanism of "Sirba" is based is autosomal in "Nile".

The evolution of sex chromosomes in "Sirba" is basically similar to that discussed in the other species. An interesting additional feature of "Sirba" is the indication from sex chromosomes of the existence of two "subsiblings" which interbreed rarely if at all. These appear identical in banding pattern and floating inversions. They differ in that the X chromosome of one is based on the standard sequence, that of the other on the inversion IS-3.

The absence of a sex linked inversion in one subsibling further illustrates the principle that an inversion by itself does not determine sex but enhances the primary mechanism.

Strangely, all sex linked inversions are located on different chromosomes. A similar observation has been reported by Dunbar (1969) in the "Sanje" subgroup where sex chromosomes of "Sanje" are located on the IIIS arm, in "Sebwe" on C_{III} and in "Kagera" on the IIL arm.

Among related species, it would seem more usual to find sex loci located on a common chromosome arm though represented by different rearrangements as reported by Rothfels (1956) in Prosimulium species and by Landau (1962) in the S. tuberosum species group; however in P. onychodactylum siblings, Newman (1972) has reported sex chromo-

somes on two different chromosome arms. Ottonen et al (1969) and Bedo (1973) have reported similar observations in P. multidentatum and S. pictipes respectively. Thus the S. damnosum situation is not unique.

The sharing of rearrangements among species appears curious.

IIL-C.8 is fixed in both "Sirba" and "Dieguera" which are in the same lineage. It has already been pointed out that it is a sex chromosome in "Nile". "Dieguera" is remarkable in other respects because IS-2 which floats in "Bille", "Nile" and "Sirba" has become fixed in "Dieguera". Similarly IL-2 which floats in "Yah" also characterizes homozygously all available specimens of "Dieguera".

In the "Sanje" subgroup, Dunbar (1969) has reported similarly that a floating inversion in "Nyamagasani" has become fixed in "Nkusi".

"Nyamagasani" is chosen, as a convenient standard for both the "Sanje" and "Nile" subgroups. It is however, not necessarily considered the progenitor of these subgroups. As regards the "Nile" subgroup, the presence of shared arrangements among species, the autosomal state of certain rearrangements in some species but the sexual differentiation of the same rearrangements in others is suggestive of common ancestry. The absence of observable sex chromosomes among some dipterans as reported by Martin (1969) in Chironomus oppositus Madahar (1969) in some siblings of Stegopterna and Dunbar (1969) in some members of the "Sanje" subgroup, suggests that the evolution of distinctive

sex mechanisms probably occurred after the diversification of species. On this basis it is assumed that the "Nile" subgroup evolved from primodial, polymorphic population with individuals carrying IS-2 and IL-2 as some of the polymorphs. Rearrangements other than these were probably derived as speciation proceeded.

Some of the ancestral polymorphs have been retained in different lineage groups of present day populations.

The standard constituent of IIIL-2 is a characteristic of the "Bille"-Yah" line, the inverted constituent still rarely floats in the "Bandama-Soubre" pair. But the inversion has become fixed in the "Nile-Sirba-Dieguera" line. Similarly IS-2 and IL-12 are carried intraspecifically in some species, interspecifically in others.

The possible alternative that the sharing of rearrangements among species suggests recent introgression appears ruled out because of the absence of active hybridization among them. "Yah" x "Bandama" hybridization is known from two individuals, which is very rare considering the total number of individuals studied. It is usual to find occasional hybrids in related dipteran species as reported by Dobzhansky (1973) for Drosophila pseudoobscura and D. persimilis, Dunbar and Vajime (1972) for "Nyamagasani" and "Sebwe" and by Ottonen et al in P. multidentatum.

Accepting that ideal biological species do not hybridize in nature and in view of the shared chromosome rearrangements between the sibling pairs investigated, it is suggested that on cytological grounds, "Bille", "Yah", "Bandama", "Soubre", "Nile", "Sirba" and "Dieguera" represent discrete taxa within the S. damnosum complex.

APPENDIX

TABLE 10

Collection Data

Location		Date	Total Larvae Collected	Collector
Bandama Valley				
1. Tiassale	Ivory Coast	14.4.66	34	B.P.
2. Tiassale	Ivory Coast	25.4.68	28	B.P.
3. Tiassale	Ivory Coast	23.6.71	3	Y.S., C.G.V.
4. Ahouati	Ivory Coast	23.6.71	98	Y.S.
5. Taabo	Ivory Coast	24.6.71	132	Y.S.
6. Zougoussou	Ivory Coast	17.6.71	3	Y.S., C.G.V.
7. Beoumi	Ivory Coast	15.6.71	89	Y.S., C.G.V.
8. Marabadiassa	Ivory Coast	16.6.71	14	Y.S., C.G.V.
9. Niakaramandougou	Ivory Coast	15.6.71	88	Y.S., C.G.V.
10. Badikaha	Ivory Coast	15.6.71	88	Y.S., C.G.V.
11. Korhogo	Ivory Coast	15.6.71	50	Y.S., C.G.V.
12. Korhogo	Ivory Coast	14.6.71	18	B.P.
Komoe Watershed				
13. Leraba Bridge	Upper Volta/Ivory Coast	20.6,67	12	B.P.
14. Leraba Bridge	Upper Volta/Ivory Coast	10.6.71	118	C.G.V.

TABLE , 10 (Cont'd)

Location		Date	Total Larvae Collected	Collector
Volta River System				
15. Bougouri-Ba, Nabere Area	Upper Volta	7.6.71	10	B.P., C.G.V.
16 Black Volta at Guena	Upper Volta	3.6.71	11	B.P., C.G.V.
17. Black Volta at Chute Dienkoa	Upper Volta	8.6.71	60	B.P., C.G.V.
18. Black Volta at Banzo	Upper Volta	11.6.71	33	B.P., C.G.V.
19. R. Piandi at Lanviera	Upper Volta	9.6.71	13	B.P., C.G.V.
20. Black Volta at Samandeni	Upper Volta	14.6.71	29	B.P., C.G.V.
21. Black Volta at Samandeni	Upper Volta	15.6.71	25	B.P., C.G.V.
22. Black Volta at Transilla-Meme	Upper Volta	22.4.66	34	B.P.
23. Black Volta at De Dougou-Nouna	Upper Volta	13.6.71	19	B.P.
24. Black Volta at De Dougou-Nouna	Upper Volta	7.6.71	89	C.G.V.
25. Black Volta at De Doutou-Tougan	Upper Volta	8.6.71	33	B.T., C.G.V.
26. White Volta, R.Sisili Nakon area, Ghana	Ghana	2.7.71	30	B.T. ,C.G.V.
27. White Volta, R. Nasia, Nasia area, Ghana	Ghana	3.7.71	104	B.T., C.G.V.
28. Red Volta, Nangodi area,	Ghana	5.7.71	72	B.T.,C.G.V.
29. White Volta, Vea River at Vea Dam		24.10.71	38	F.W.
30. White Volta, near Akoaombo Dam, Ghana		11.7.71	10	F.W., C.G.V.

TABLE 10 (Cont'd)

Location	Date	Total Larvae Collected	Collector
River Niger System			
31. R. Iku at Abuja, Nigeria	14.7.69	36	C.G.V.
32. R. Iku at Abuja, Nigeria	6.8.71	9	C.G.V.
33. R. Kontagora at Mokwa-KainjiBridge, Nigeria	9.8.71	2	S.A.O., C.G.V.
34. R. Oli at Kainji, Nigeria	10.8.71	31	S.A.O., C.G.V.
35. R. Mayo Boki, Nagaoundere area, Cameroon	27.7.68	81	R.H.L.D.
36. R. Mayo Salah Nagaoundere area, Cameroon	30.7.68	55	R.H.L.D.
37. R. Sirba near Kwarezenou, Niger	5.10.69	30	R.L.
38. R. Niger at Sotuba dam, Mali	2.6.71	7	B.P., C.G.
39. R. Banifing at Kouoro, Mali	2.6.71	33	B.P., C.G.V.
River Senegal System			
40. R. Bafing at Dieguera, Mali	18.5.71	24	B.P.
41. R. Senegal at Gouina, Mali	17.5.71	26	B.P.
42. R. Papara at Gue, Mali	28.8.71	5	B.P.
43. R. Papara at Chute Papara, Mali	28.8.71	11	B.P.

TABLE 10 (Cont'd)

Location	Date	Total Larvae Collected	Collector
River Sassandra System			
44. Sassandra at Soubre, Ivory Coast	18.6.71	73	Y.S.C.G.V.
45. Sassandra at Soubre, Ivory Coast	4.5.68	35	B.P.
46. Zozola Stream near Soubre, Ivory Coast	18.6.71	97	Y.S., C.G.V.
47. Zordo near Issia, Ivory Coast	4.5.68	15	B.P.
48. R. Zo at Danane-Man Road, Ivory Coast	22.6.71	56	Y.S., C.G.V.
River Cavally System			
49. R.Nze at Tai, Ivory Coast	19.6.71	26	Y.S., C.G.V.
50. R. Cavally at Toulepleu, Ivory Coast	19.6.71	33	Y.S., C.G.V.
51. R. Cavally at Oua, Ivory Coast	20.6.71	120	Y.S., C.G.V.
52. R. Cavally, Nyaake area, Liberia	12.6.71	121	R.G.
River Cestos System			
53. R. Tien near Danane-Liberia Rd., Ivory Coast	21.6.71	73	Y.S., C.G.V.
54. Yeal Streams of Mt. Nimba, Ivory Coast	21.6.71	4	Y.S., C.G.V.
55. Yah Creek, Yekepa Nimba County, Liberia	10.6.70	99	R.G.
56. Cestos River near Darlu, Liberia	6.5.71	75	R.G.
57. Cestos River, Liberia	16.6.71	88	R.G.
58. Cestos River, Liberia	12.5.71	37	R.G.

TABLE 10 (Cont'd)

Location	Date	Total Larvae Collected	Collector
St. John Watershed			
59. St. John R., Soko Town, Nimba County, Liberia	1.3.71	38	R.G.
60. St. John R., Duo Town, Nimba County, Liberia,	1.3.71	10	R.G.
61. Blaygbi Creek, Grand Bassa County, Liberia,	11.3.71	20	R.G.
62. Blaygbi Creek, Grand Bassa County, Liberia,	12.4.71	36	R.G.
63. Blaygbi Creek, Grand Bassa County, Liberia,	9.7.71	49	R.G.
64. Blaygbi Creek, Grand Bassa County, Liberia,	26.5.71	49	R.G., C.G.V.
Farmington Watershed			
65. Farmington R., Firestone Plantation, Liberia	24.5.71	32	D.W., C.G.V.
66. Du R., Firestone, Plantation, Liberia	24.5.71	3	R.G.
67. Du-plimo, Firestone Plantation, Liberia	3.7.71	108	R.G.
68. Numoni Creek, Firestone Plantation, Liberia	24.5.71	50	D.W., C.G.V.
69. Borlo Creek, Liberia,	29.5.71	7	R.G.

TABLE 10 (Cont'd)

Location	Date	Total Larvae Collected	Collector
St. Paul Watershed			
70. St. Paul R., Dubli Island, Bong County, Liberia	8.10.68	10	R.G.
71. St. Paul R., Dubli Is., Bong County, Liberia	7. 4.69	18	R.G.
72. St. Paul R., Near Belefuani, Liberia,	8. 4.69	34	R.G.
73. St. Paul R., Dubli Is. Bong County, Liberia	12.5.69	12	R.G.
74. St- Paul R., Dubli Is. Bong County, Liberia	25. 5.69	50	R.G.
75. St. Paul R., Lofa County, Liberia,	25. 3.71	23	R.G.
76. St. Paul R., Dubli Is., Bong County, Liberia	23. 3.71	41	R.G.
77. St. Paul R. Dubli Is., Bong County, Liberia	7. 4.71	20	R.G.
78. St. Paul R., Dubli Is., Bong County, Liberia	7. 4.71	16	R.G.
79. St. Paul R., Dubli Is., Bong County, Liberia,	19. 5.71	79	R.G., C.G.V.
80. Sia R., Lofa County, Liberia	13.11.69	44	R.G.
81. Mein R., Montserrado County, Liberia	25. 6.70	30	R.G.
82. Waterfall, Bong Hill, Liberia	19. 5.71	40	R.G., C.G.V.
83. Waidei Creek, Bong Area, Liberia	19. 5.71	108	R.G., C.G.V.
84. Marvo Creek, Bong Area, Liberia	19. 5.71	11	R.G., C.G.V.
85. Marvo Creek, Bong Area, Liberia	10. 7.71	54	R.G., C.G.V.

TABLE 10 (Cont'd)

Location	Date	Total Larvae Collected	Collector
Lofa Watershed			
86. Lofa R., Lofa County, Liberia	9.7.71	52	R.G.
87. Wolejai Creek, near Voinjama, Liberia	8.4.69	41	R.G.
88. Garbayea Creek, Lofa County, Liberia	8.4.69	11	R.G.
89. Wuanwo Creek, Lofa County, Liberia	21.3.71	37	R.G.
90. Harlayah Creek, Lofa County, Liberia	23.3.71	116	R.G.
91. Wolejai Creek, Lofa County, Liberia	23.3.71	21	R.G.
92. Garbayea Creek, Lofa County, Liberia	24.3.71	81	R.G.
Mano Watershed			
93. Mano River, Liberia	17.7.71	39	R.G.
94. Bendaja Creek, Liberia	13.7.71	61	R.G.
95. Gbarpi Creek, Liberia	24.6.71	17	R.G., C.G.V.

TABLE 10 (Cont'd)

Location	Date	Total Larvae Collected	Collector
Mungo Watershed			
96. Blackwater R., Ebonje Area, Cameroon	8.6.68	93	R.H.L.D.
97. Blackwater R., Ebonje Area, Cameroon	6.6.68	6	R.H.L.D.
98. Blackwater R., Ebonje Area, Cameroon	4.6.68	23	R.H.L.D.
99. Blackwater R., Ebonje Area, Cameroon	17.3.69	27	R.H.L.D.
100. Vina R., Wakwa Area, Cameroon	4.6.68	42	R.H.L.D.
Meme Watershed			
101. Bille R., Kumba Area, Cameroon	29.8.68	38	R.H.L.D.
102. Bille R., Kumba Area, Cameroon	5.3.69	139	R.H.L.D.
103. Bille R., Kumba Area, Cameroon	12.1.69	105	R.H.L.D.
104. Bille R., Kumba Area, Cameroon	14.8.71	75	C.G.V.
105. Bille R., Kumba Area, Cameroon	16.8.71	25	C.G.V.

Collectors :

R.H.L. Disney
B.O.L. Duke
R. LeBerre
R. Garms
S.A. Oyewole
B. Philippon
Y. Sechan
B. Thompson
C.G. Vajime
F. Walshs
D. Williams

REFERENCES

1. Basrur, P.K. 1959. The Salivary gland chromosomes of seven segregrates of Prosimulium (Diptera: Simuliidae) with a transformed centromere. Can.J.Zool. 37, 527-570.
2. Bedo, D. 1973. Sibling species and sex chromosomes in black files of The Simulium pictipes group (Diptera: Simuliidae). M.Sc. Thesis, Botany Dept. Univ. of Toronto.
3. Blacklock, D.B. 1926. The development of Onchocerca volvulus in Simulium damnosum. Ann. trop. med. Parasit. 20, 1.
4. Beerman, W. and U. Clever. 1964. Chromosome Puffs. Sc.Amer. April.
5. Berendes, H.D. 1968. Factors involved in the Expression of Gene Activity in Polytene Chromosomes. Chromosoma (Berl.) 24, 418-437.
6. Bryan, H., M. Colluzzi, 1971. Cytogenetic observations on Anopheles farauti Laveran. Bull.Wld. Hlth. Org. 45 266-267.
7. Cassagnau, P. 1971. Les Chromosomes Salivaires Polytenes Chez Bilobella grassei (Denis) (Collemboles: Neanuridae) Chromosoma (Berl.). 35, 57-83.
8. Chandler, A.C. and C.P. Read 1967. Introduction to Parasitology. With special reference to the parasites of man. 10th Edition. John Wiley & Sons, Inc. New York.
9. Colluzzi, M and Sabatini, 1967. Cytogenetic observations on species C of the Anopheles gambiae complex. Parasitologia. 9, 73-88.

10. Colluzzi, M and A. Sabatini, 1968. Cytogenetic observations on species C of the Anopheles gambiae complex. Parasitologia. 10, 155-165.

11. Colluzzi, M and A. Sabatini, 1968. Cytotaxonomic identification of the species of Anopheles gambiae complex. 8th Intern. Congress Trop. Med. Malar. Teheran-Iran.

12. Colluzzi, M and A. Sabatini, 1969. Cytogenetic observations on the salt-water species Anopheles merus and Anopheles melas of the gambiae complex. Parasitologia. 11, 177-187.

13. Crisp, G. 1956. Simulium and onchocerciasis in the Northern Territories of the Gold Coast. H.K. Lewis & Co., Ltd. London.

14. Crosskey, R.W. 1969. A re-classification of the Simuliidae (Diptera) of Africa and its islands. Bull Brit. Mus. (Nat. Hist. Ent.) Suppl. 14.

15. Davies, J.B. 1962. Egg-laying habits of Simulium damnosum Theobald and Simulium medusaeforme from Hargrevesi Gibbins in Northern Nigeria. Nature. 196, 149-150

16. Davies, J.B. 1963. Further distribution records of the black flies (Diptera: Simuliidae) of Nigeria with notes on the occurrence of Simulium damnosum Theo. in abnormal situations J. W.Afr. Sc. Assoc. 7, 134-137.

17. Davies, J.B. 1968. The Simulium Control Scheme at Abuja, Northern Nigeria, and its Effect on the Prevalence of Onchocerciasis in the Area. Bull. Wrd. Hlth. Org. 39, 187-207.

18. Disney, R.H.L. and P.F.L. Borcham. 1969. Blood gorged resting black flies in Cameroon and evidence of Zoophily in Simulium damnosum. Trans. Roy. Soc. trop. Med. Hyg. 63, 286-287.

19. Disney, R.H.L. 1969. A note on variation within Simulium damnosum in the forest zone of West Cameroon. Ann. trop. Med. Parasit. 64, 129-130.

20. Dobzhansky, T. 1972. Species of Drosophila. Science, 177 664-669.

21. Dobzhansky, T. 1973. Is there gene exchange between Drosophila pseudoobscura and Drosophila persimilis in their natural habitat? Amer. Nat. 107, 161-320

22. Duke, B.O.L. D.J. Lewis, and P.J. Moore., 1966. Onchocerca Simulium complexes. I. -- Transmission of forest and Sudan-Savannah strains of Onchocerca volvulus, from Cameroon, by Simulium damnosum from various West African bioclimatic zones. Ann. trop. Med. Parasit. 60, 318-336.

23. Dunbar, R.W. 1959. The salivary gland chromosomes of seven forms of black flies included in Eusimulium aureum. Fries. Can. J. Zool. 37, 495-525.

24. Dunbar, R.W. 1966. Cytotaxonomic studies in black flies (Diptera: Simuliidae). "Chromosomes Today" I, 179-181.

25. Dunbar, R.W. 1966. The salivary gland chromosomes of six closely related black flies near Eusimulium congareenarum (Diptera: Simuliidae). Can. J. Zool. 45, 377-396

26. Dunbar, R.W. 1966. Four sibling species included in Simulium damnosum Theobald (Diptera: Simuliidae) from Uganda. Nature (Lond), 209, 597-599

27. Dunbar, R.W. 1969. Nine Cytological segregates in the Simulium damnosum complex (Diptera: Simuliidae). Bull. Wld. Hlth Org. 40 , 974-979

28. Dunbar, R.W. 1972. Polytene chromosome preparations from tropical Simuliidae. WHO Mimeographed Document: WHO/ONCHO/72.95

29. Dunbar, R.W. and C.G. Vajime, 1972. The Simulium (Edwardsellum) damnosum complex. A report on cytotaxonomic studies to April 1972. WHO Mimeographed Document: WHO/ONCHO/72.100.

30. Garms, R and A. Post, 1966 Die Verbreitung von Simulium damnosum in Guinea/West Africa. Tropenmed. Parasitol. 17, 443-466

31. Garms, R and F. Weyer, 1968. Naturliche Infektion von Simulium damnosum mit. Onchocerca Volvulus in Savannengebieten Guineas Tropenmed. Parasitol 19, 289-296.

32. Garms, R. and J. Voelker, 1969. Unknown Filarial larvae and Zoophily in Simulium damnosum in Liberia. Trans. Roy Soc. trop. med. Hyg. 63, 676-677.

33. Garms, R. 1973. Zur Verbeitung von Simulium damnosum in Liberia Z. tropenmed. Parasitol 24, 222-231.

34. Germain, M. , P. Grenier and J. Mouchet, 1968. Les Simulies du Cameroun Occidental. Influence du milieu physique sur leur repartition. Cash. ORSTOM ser. Ent. med. VI, 167-190.

35. Kitzmiller, J.B. , G. Frizzi and R.H. Baker, 1967. Evolution and speciation within the Maculipennis complex of the Genus Anopheles: in "Genetics of Insect Vectors of Disease" by Wright, J.W., and R. Pal. Elsevier Pub. Co.

36. Knuttgen, H.J., 1964. Untersuchungen uber Vorkommen and Bedeutung der Onchozerkose in Guinea, West Africa. Z. Tropenmed. Parasitol. 15, 427-435.

37. Landau, R. 1962. Four forms of Simulium tuberosum (Lundstr) in Southern Ontario. A salivary gland chromosome study. Can. J. Zool. 40, 921-939.

38. Le Berre, R; G. Balay, J. Brengues and J. Coz. 1964. Biologie et ecologie de la femelle de Simulium damnosum Theobald 1903, en fonction des zones bioclimatiques d'Afrique occidentale. Influence sir ;'epidemiologie de l'oncho-cercose. Bull. Org. Mond. Sante, 31, 843-855.

39. Le Berre, R. 1966. Contribution a l'etude biology et ecologique de Simulium damnosum Theo. 1903 (Diptera, Simuliidae). O.R.S.T.O.M. Paris.

40. Lewis, D.J. and B.O.L. Duke. 1966. Onchocerca Simulium complexes. II.- variation in West African female Simulium damnosum. Ann. trop. Med. Parasit 60 337-346.

41. Madahar, D.P. 1969. The salivary gland chromosomes of seven taxa in the subgenus Stegopterna (Diptera, Simuliidae, Cnephia). Can. J. Zool. 47, 115-119.

42. Martin, J. 1968. The salivary gland chromosomes of Chironomus oppositus Walker (Diptera: Nematocera) Aust. J. Zool. 17 473-486.

43. McCrae, A.W.R. 1969. Consideration of Simulium damnosum Theo. as a species complex and its relevance to the control of onchocerciasis. Report: East African Virus Research Inst., Entebbe, Uganda.

44. Merighi, B. and A.E. Parrinello. 1969. Presenza di Simulium damnosum Th. (Diptera-Simuliidae) in Yemen. Ann. 1st Super. Sanita. 5, 197-199.

45. Newman, L. 1972. Sibling species of Prosimulium onychodactylum. Unpublished document.

46. Noamesi, G.K. 1964. The breeding places in Ghana of Simulium damnosum Theobald. Ghana J. Sc. 4, 44-51

47. Ottonen, P.O. and R. Nambiar. 1969. The salivary gland chromosomes of Prosimulium multidentatum Twinn and three forms included in Prosimulium magnum (Dyar and Shannon) (Diptera: Simuliidae). Can. J. Zool. 47, 943-949.

48. Puri, I.M. 1925. On the life history and structure of the early stages of Simuliidae (Diptera, Nematocera). Part I. Parasitology 17, 295-334.

49. Rothfels, K.H. and R.W. Dunbar. 1953. The Salivary gland chromosomes of the black fly Simulium vittatum (Zett). Can. J. Zool. 31, 226-241.

50. Rothfels, K.H. 1956. Black flies : Siblings sex and species grouping. J. Heredity. 47, 113-122.

51. Rothfels, K.H. 1973. Sex differential segments in Eastern North American species related to Prosimulium fuscum. Unpublished document.

52. Theobald, F.V. 1903, Report on a collection of mosquitoes and other flies from equatorial East Africa and the Nile provinces of Uganda. S.S. B. London Reports 3, 33-42.

53. Wulker, W., J.E. Sublette and J. Martin, 1968. Zur cytotaxionomie Nordamerikanischer Chironomus - Arten Ann. Zool. Fennici 5, 155-158.

54. Zimring, R. 1953. A comparative study of the salivary gland chromosomes of five related black fly species. M.A. thesis. University of Toronto.

PLATE 1

Fig. 1 Male meiotic chromosomes in S. damnosum showing single chiasmata and a double chiasma (arrow).

Fig. 2 Entire salivary gland chromosome complement of larva of "Nile" sibling.

Fig. 3 Centric fusion (arrows) in "Yah".

Fig. 4 Centric ectopic pairing (arrows) in "Yah".

Legend: NO = Nucleolar Organiser

C = Centromere

db = double bubble

B = Balbiani Ring

PB = Para Balbiani

b = blister

PLATE I

PLATE 2

Salivary gland chromosome idiograms of seven species included in the S. damnosum complex from West Africa.

Fig. 5 "Bille"

Fig. 6 "Yah"

Fig. 7 "Dieguera"

Fig. 8 "Soubre"

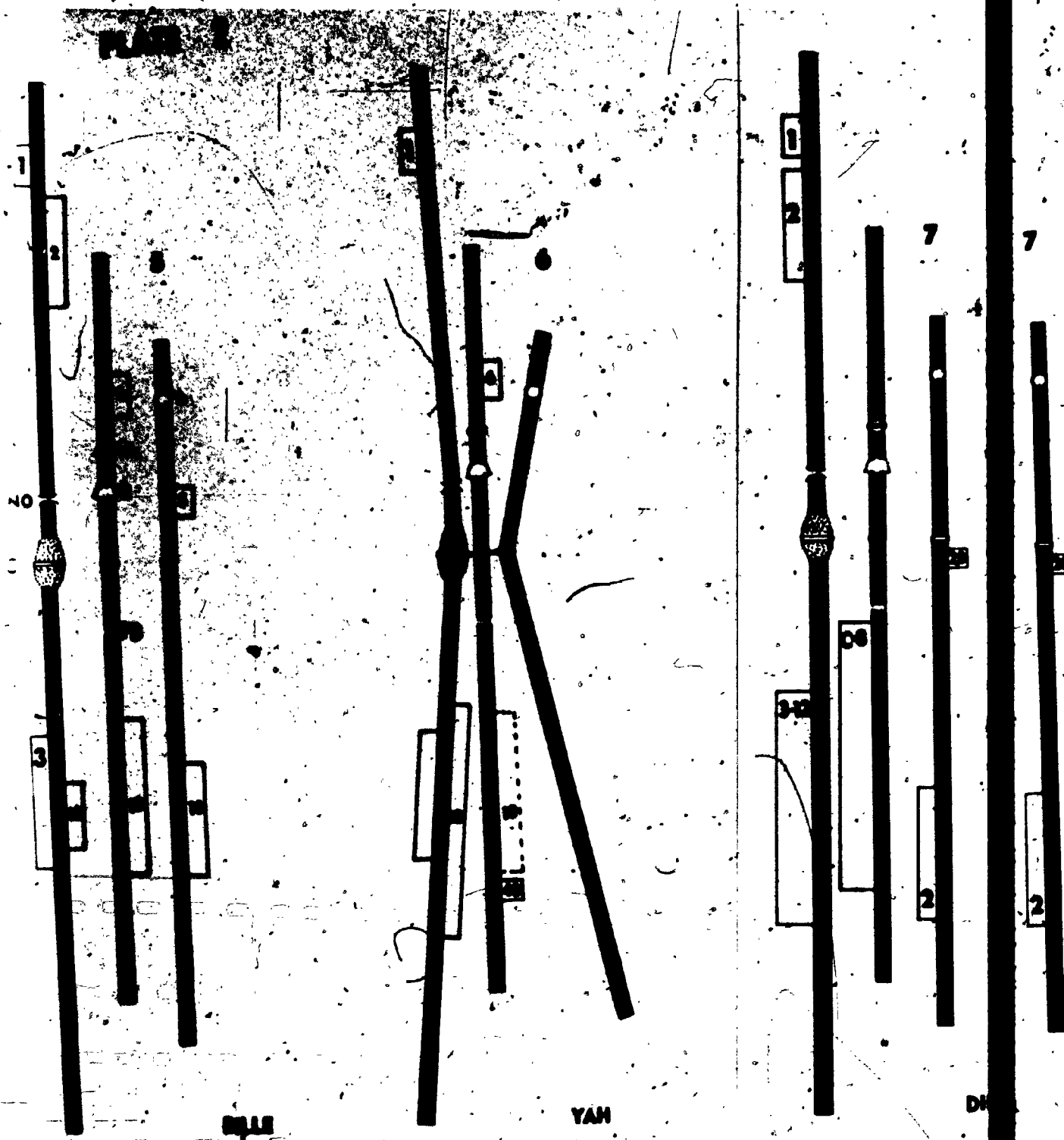
Fig. 9 "Bandama"

Fig. 10 "Nile"

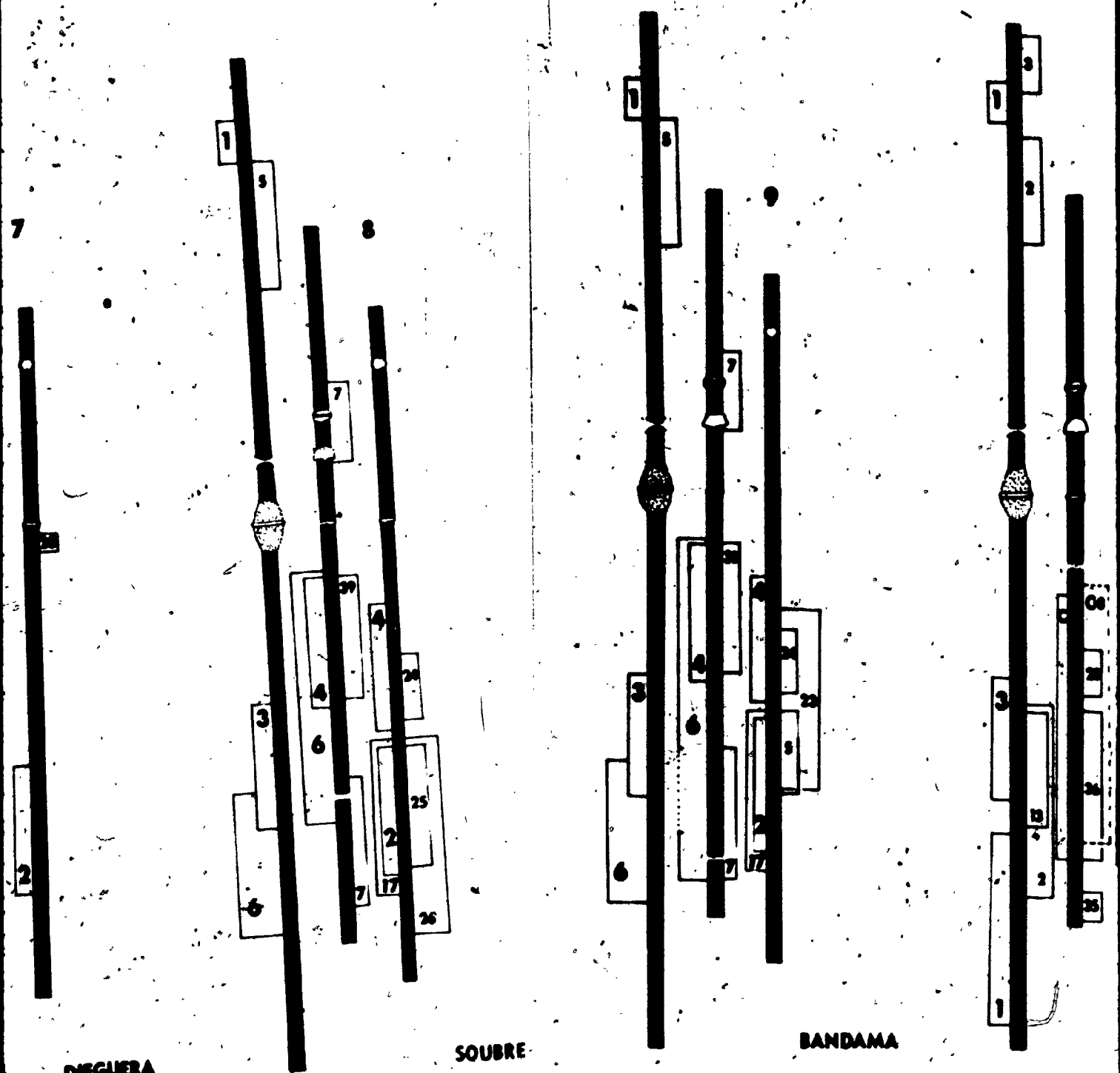
Fig. 11 "Sirba"

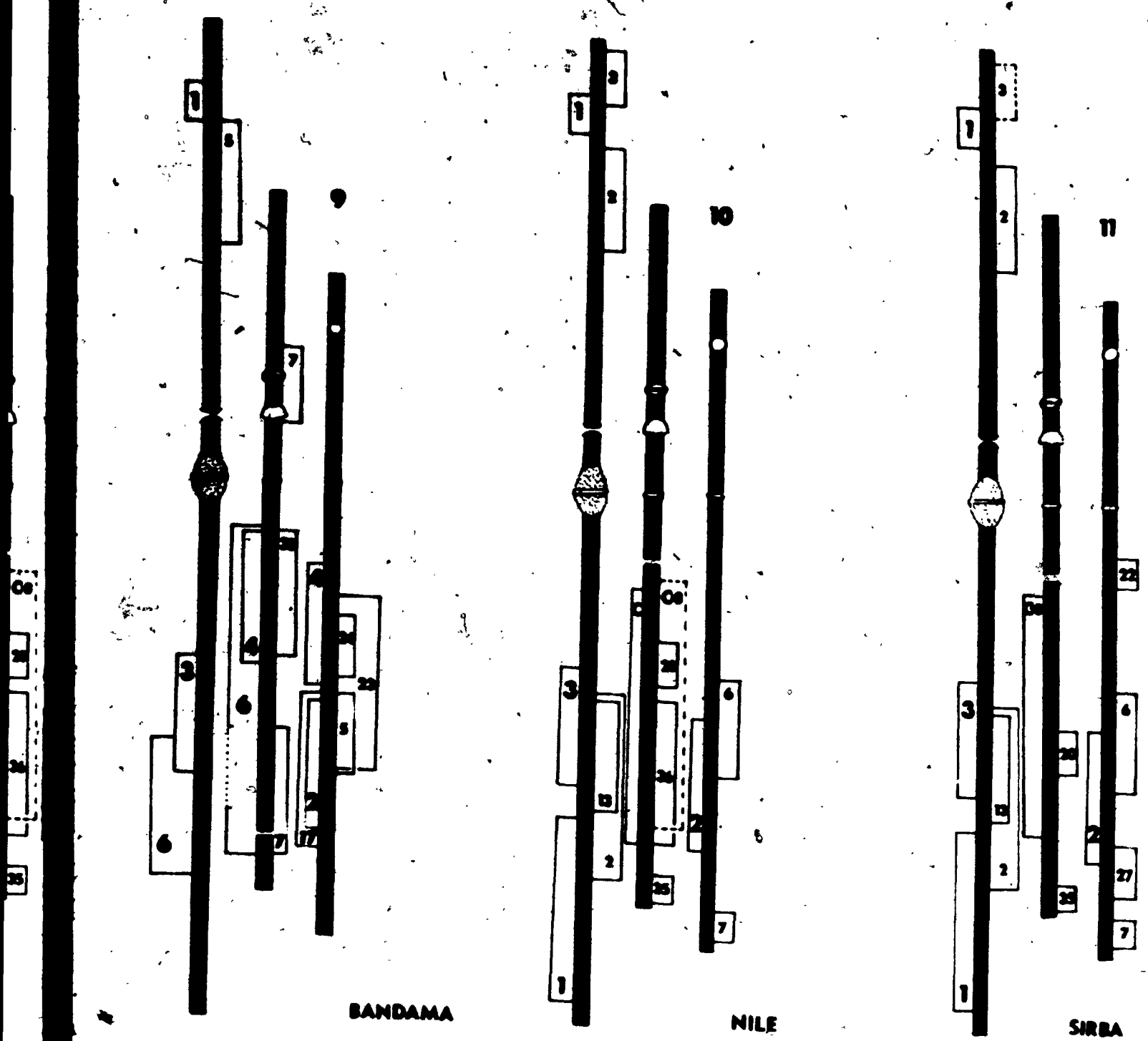
For legend refer to Plate 1. Interspecific (fixed) inversions shown by solid brackets to the left; Intraspecific (floating) inversions shown by brackets to the right; Sex inversions shown by broken brackets to the right of chromosome arms.

1 of



2 of





9

BANDAMA

10

NILE

11

SIRBA

PLATE 3

Standard IS and IS of "yah", "Bille","Bandama", "Nile" and "Sirba"
Interspecific (fixed) inversions shown by solid brackets to the left.

Intraspecific (floating) inversions by solid brackets to the right.

Abbreviations as in Plate 1.

Fig. 12 IS of "Sebwe", identical with standard "Nyamagasani". Break points of IS-1 are indicated. This is the basic sequence of all the siblings shown in the Plate.

Fig. 13 "Yah" sequence, IS-1. The floating inversions IS-11 ("Yah") and IS-2 ("Bille") are shown.

Fig. 14 "Bille" IS-1; homozygous for IS-2. This represents the basic sequence of "Sirba".

Fig. 15 "Bandama" sequence IS-1; identical with "Soubre". Limits of floating inversions are shown.

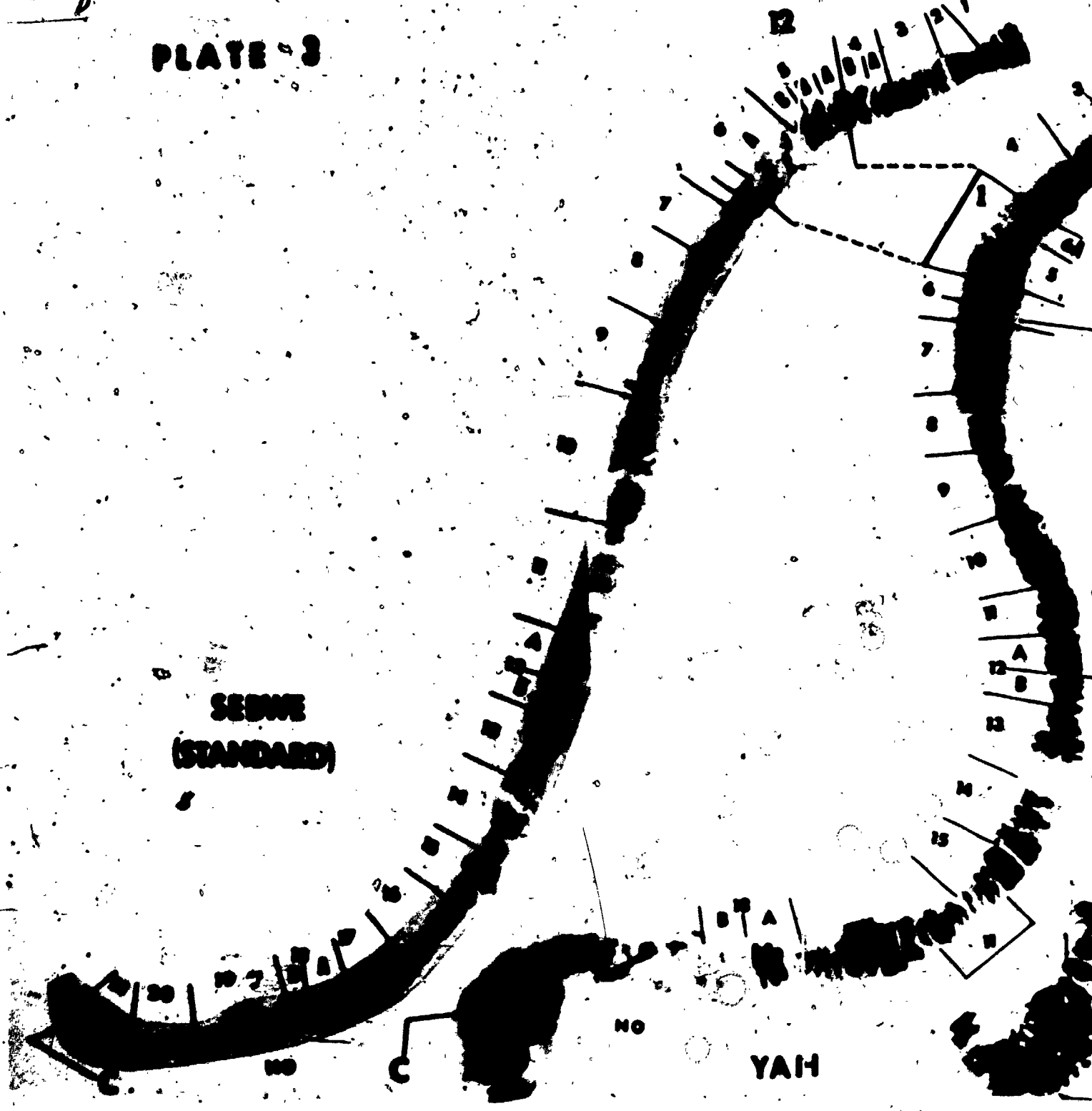
Fig. 16 "Nile" sequence, IS-1; floating inversions are shown.

Fig. 17 "Sirba" ♀, IS-1; IS-2 shown homozygously. Limits of IS-3 are marked as well to the right with broken lines. Dotted lines to the left show how IS-3 overlaps IS-1.

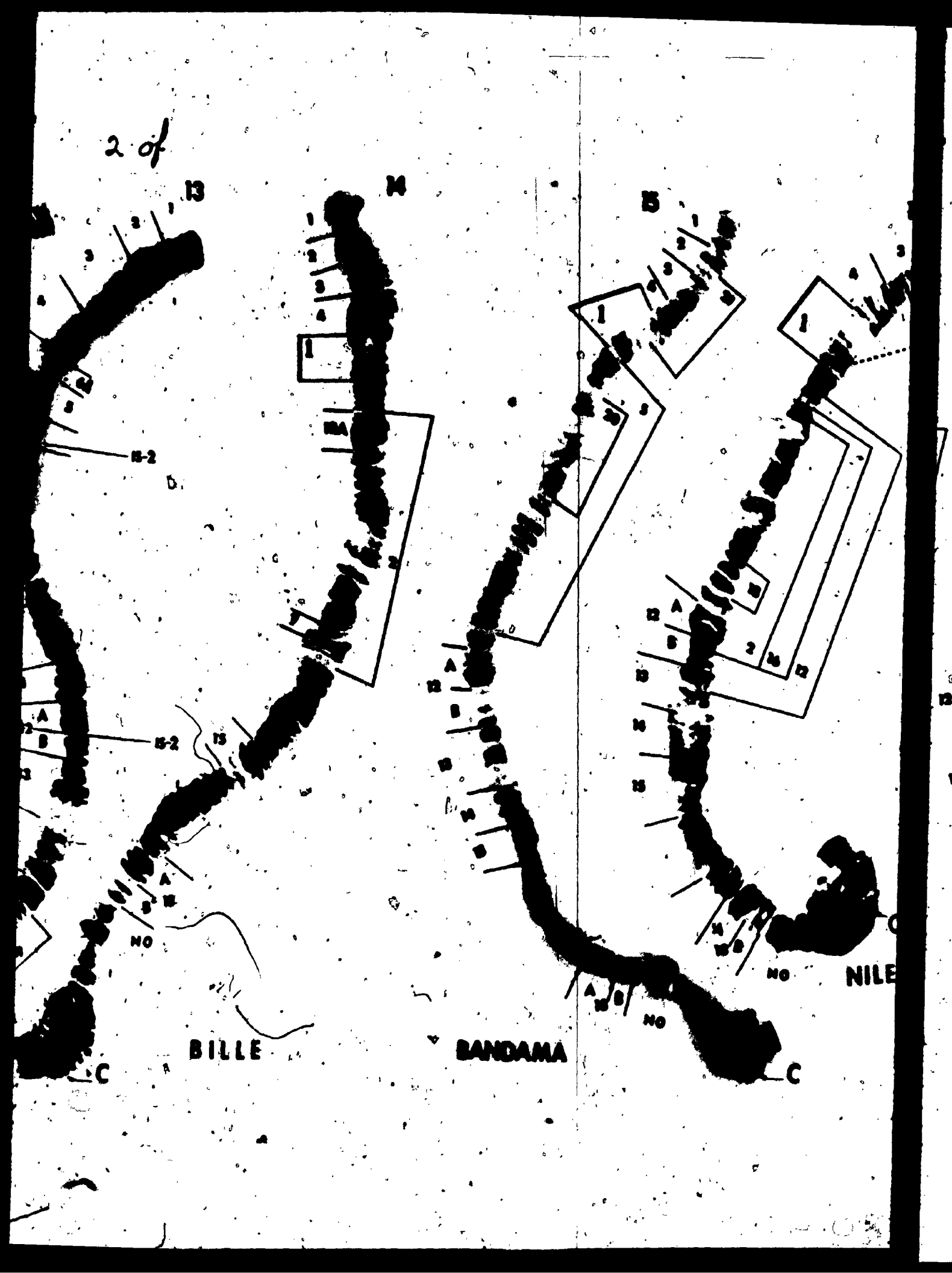
Fig. 18 "Sirba" ♂ heterozygous for IS-3, homozygous for IS-2.

1 of

PLATE 3



2 of



3 of 3

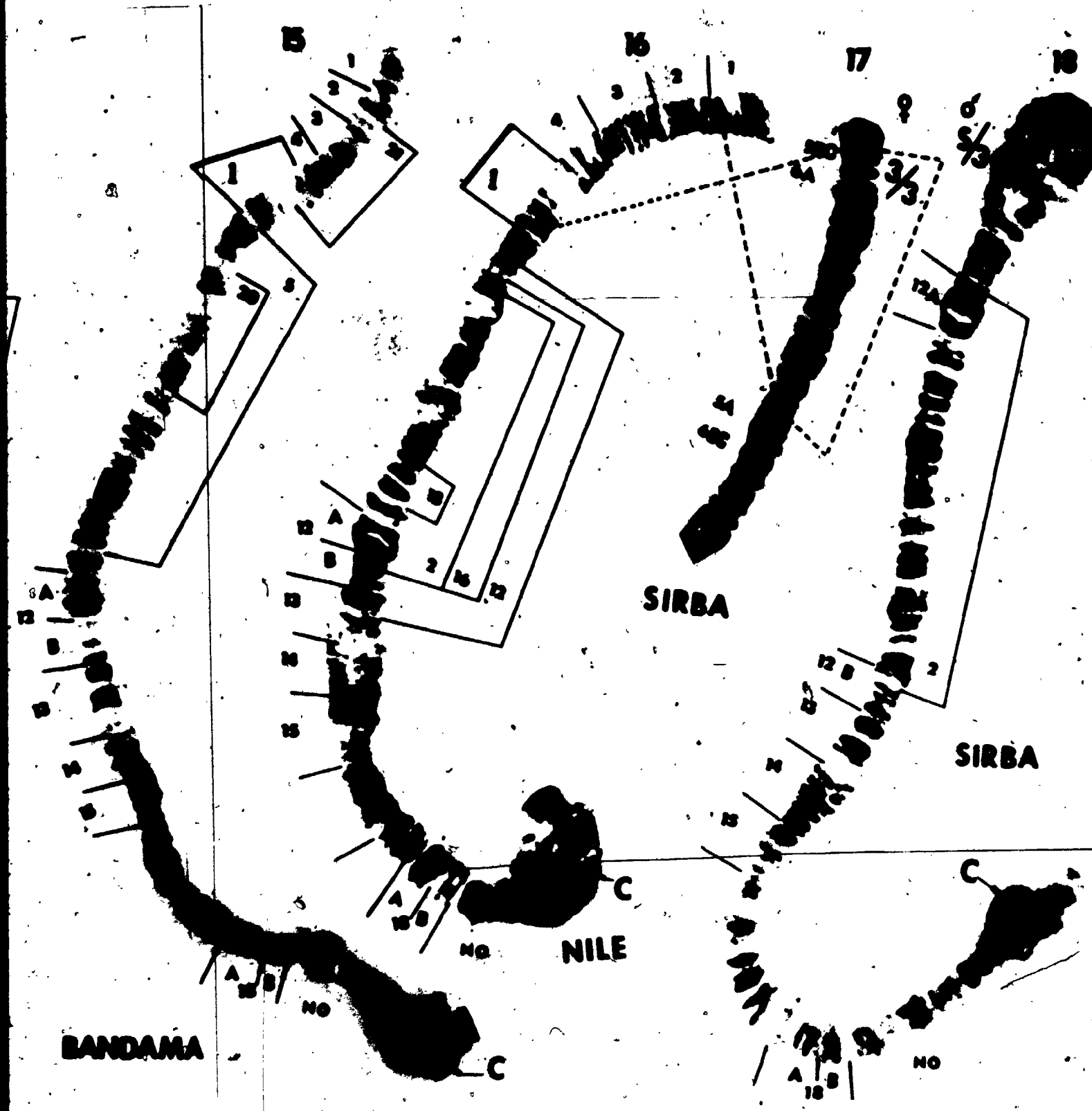


PLATE 4

IL arm

Standard IL, IL and ♂ expanded region of "Bille", plus IL "Yah" "Bandama", "Nile" and "Sirba". Interspecific (fixed) inversions are shown by solid brackets to the left, intraspecific (floating) inversions by solid brackets to the right.

Abbreviations as in Plate 1.

Fig. 19 "Sebwe" sequence identical with standard "Nyamagasani". Limits of fixed inversions IL-1 and IL-3 of other siblings are shown by lines to the right.

Fig. 20 "Bille" ♀ showing IL-3 and homozygosity for the standard arrangement of the expanded region (arrow).

Fig. 21 "Bille" ♂ showing heterozygosity for the expanded region (arrow). The Y constituent is modified as shown.

Fig. 22 "Yah" sequence, IL-3 and homozygosity for the standard arrangement of the expanded region (arrow). Limits of the floating inversions IL-12 and IL-15 are shown.

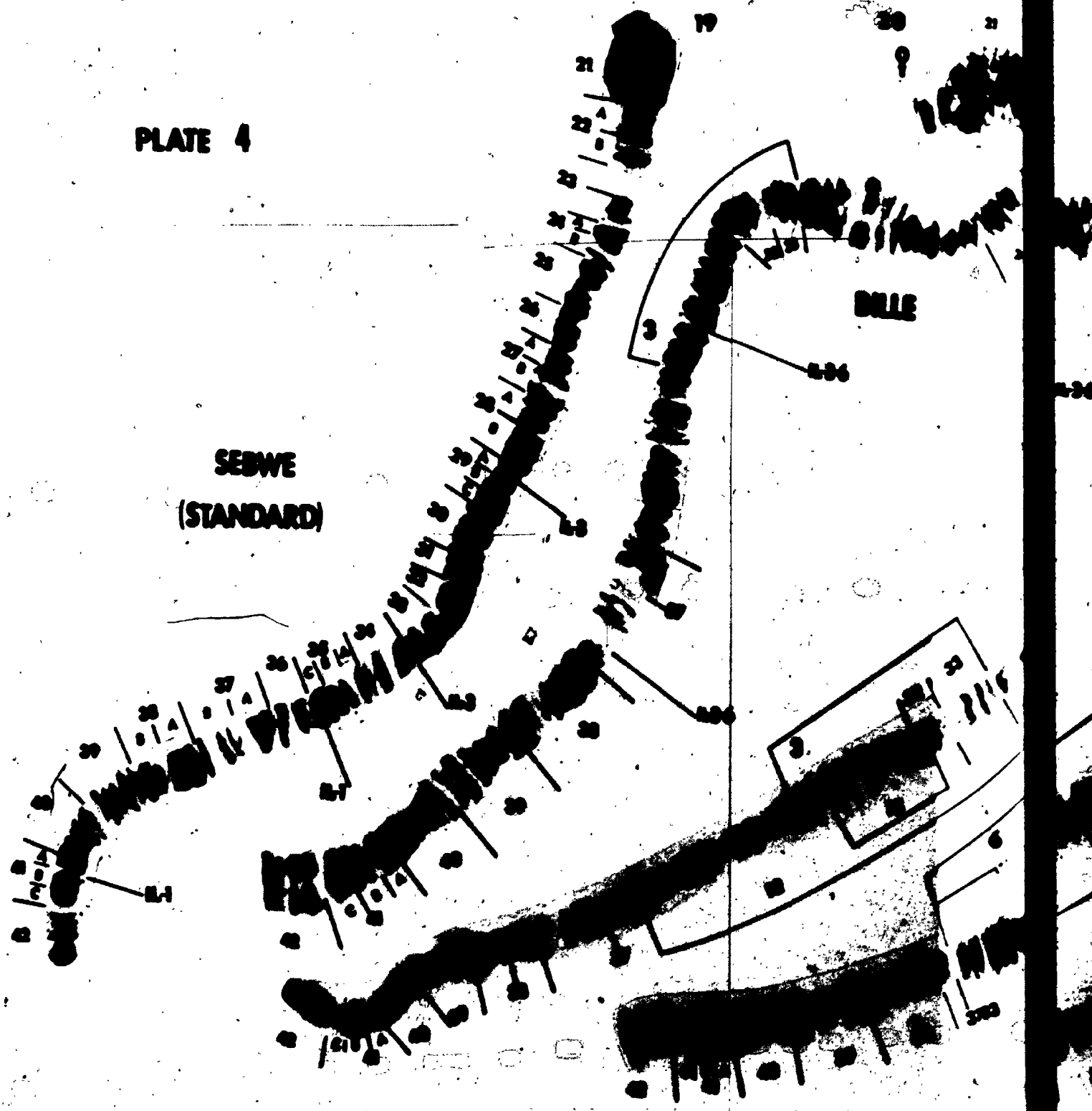
Fig. 23 "Bandama" IL-3.6 sequence is virtually fixed. "Bandama" and "Soubre" are identical in this arm.

Fig. 24 "Nile" sequence, IL-1 and IL-3. Limits of floating inversions IL-2 and IL-13 are shown.

Fig. 25 "Sirba" which is identical with "Nile" in this arm.

1 of

PLATE 4



2 of

3 of 3

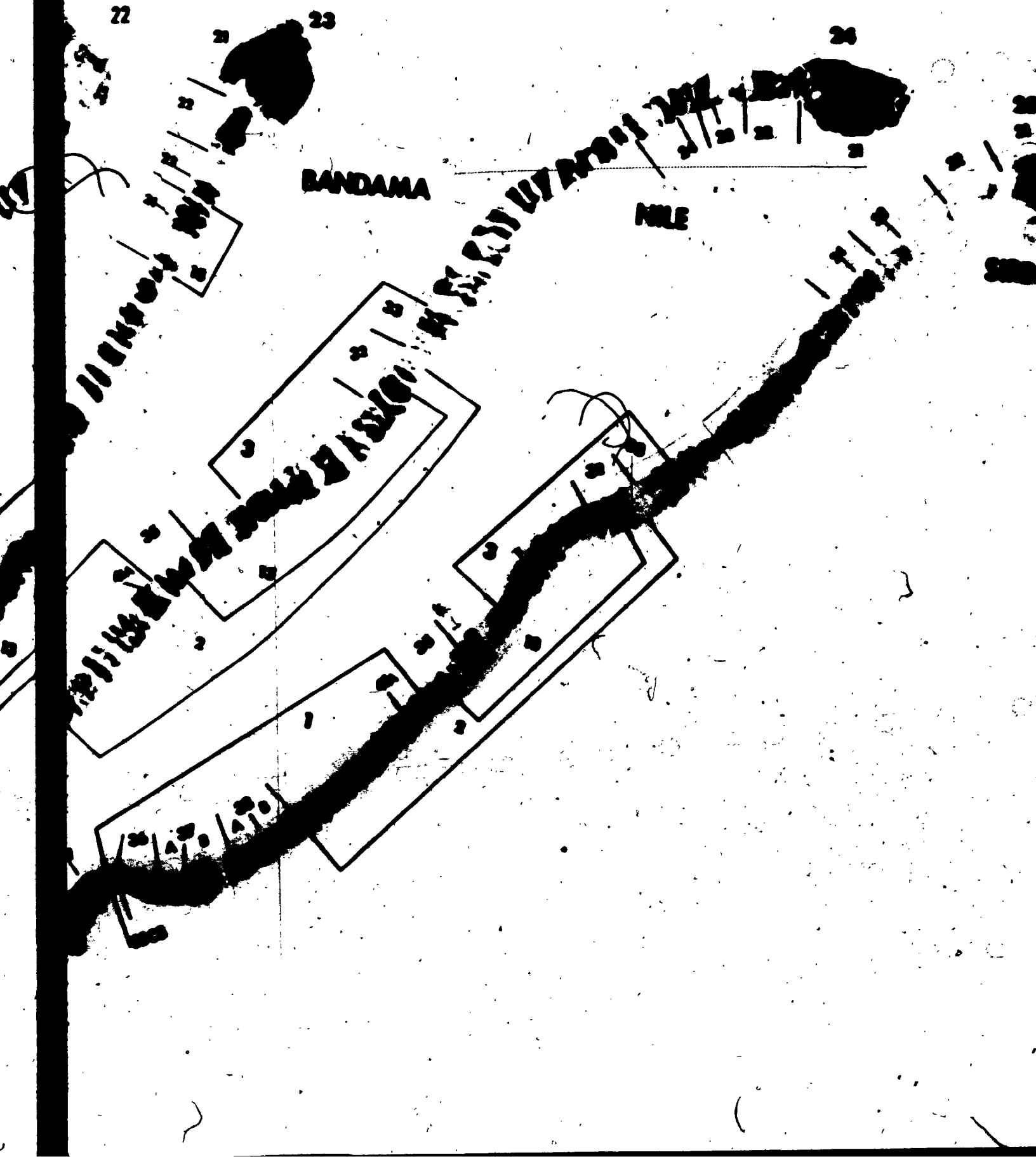


PLATE 5

Chromosome II

Chromosome II of "Bille", "Yah" and "Bandama" plus IIL arms of "Soubre". Interspecific (fixed) inversions are shown by solid brackets to the left, intraspecific (floating) inversions by solid brackets to the right.

Abbreviations as in Plate 1.

Fig. 26 "Bille" sequence, identical with standard "Nyamagasani". Limits of II L-18 ("Yah"), II L-C ("Nile", "Sirba", "Dieguera") are shown.

Fig. 27 "Yah" sequence showing homozygosity for II L-18. Limits of II L-4 ("Bandama", "Soubre") and floating inversions are shown.

Fig. 28 "Soubre" sequence showing II L-4, II L-6. Limits of II L-7 ("Bandama") are shown.

Fig. 29 "Soubre" sequence showing II L-4, II L-6 and also homozygosity for II L-39. Limits of II L-7 are shown.

Fig. 30 "Bandama" sequence, II L-6.7.
Limits of floating inversions II S-7, II L-4, 39 are shown.

1 of

PLATE 5

2 of 2

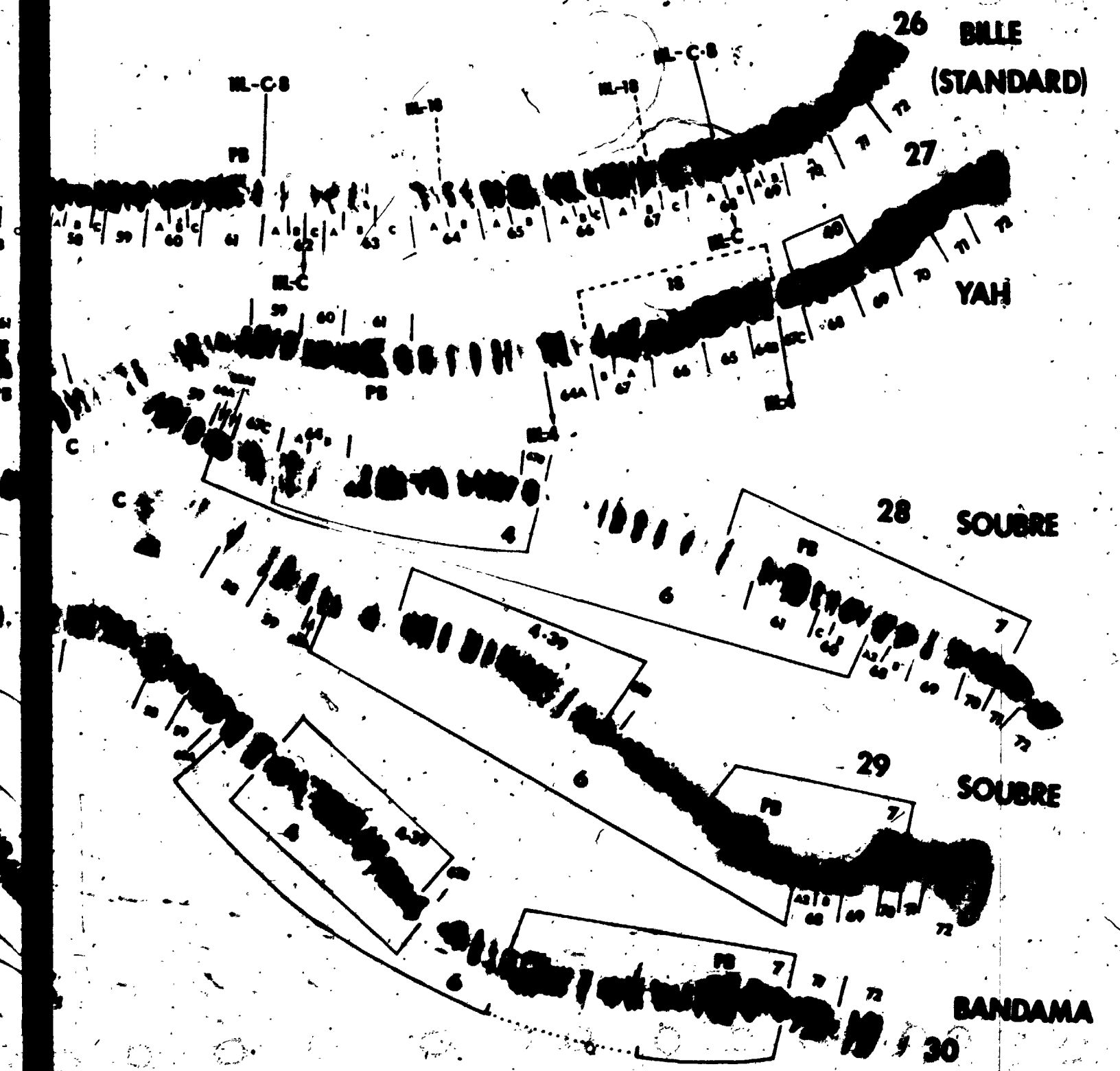

26 BILLE (STANDARD)
27 YAH
28 SOUBRE
29 SOUBRE
BANDAMA 30
IRL-C-8
IRL-10
IRL-C
IRL-A
PB

PLATE 6

Chromosome II continued

Chromosome II of "Nile" and "Sirba". Interspecific (fixed) inversions are shown by solid brackets to the left, intraspecific (floating) inversions by solid brackets to the right.

Abbreviations as in Plate 1.

Fig. 31 "Nile" sequence, II L-C, II L-C.8 (Sirba) and limits of floating inversions.

Fig. 32 "Sirba" sequence, II L-C.8, and limits of floating inversions.

Fig. 33 "Nile" ♀ homozygous for II L-C.

Fig. 34 "Nile" ♂ heterozygous for II L-C.8.

Fig. 35 "Sirba" sequence showing II L-C.8 homozygously.

Fig. 36 "Sirba" showing "exploded" "Balbiani Ring".

1 of

PLATE 6

SIRBA

2 of 2

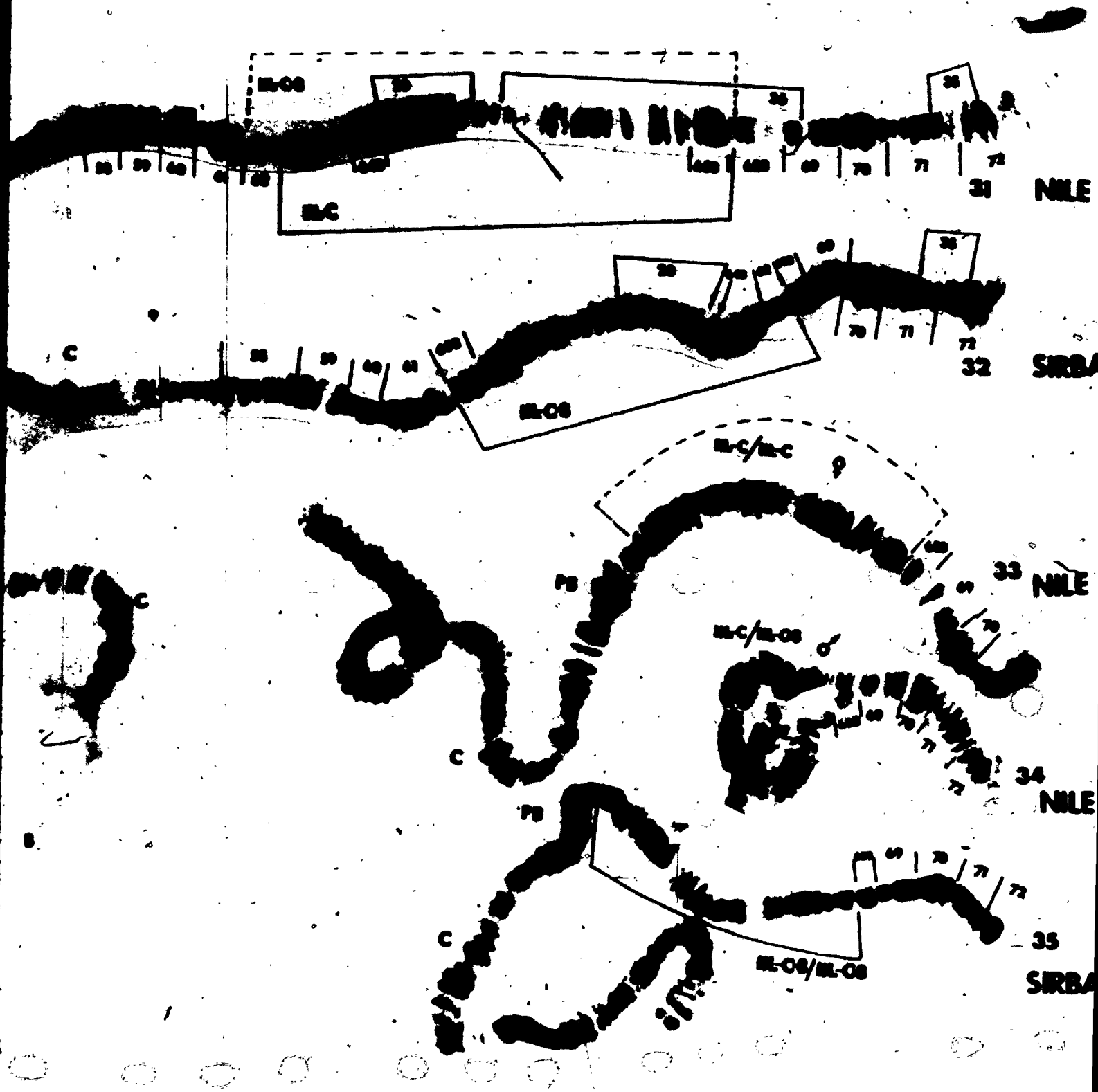


PLATE 7

Chromosome III

Chromosome III of "Yah", "Bandama" "Nile" and "Sirba". Interspecific (fixed) inversions are shown by solid brackets to the left, intraspecific (floating) inversions by solid brackets to the right.

Abbreviations as in Plate 1.

Fig. 37 "Yah" sequence, identical with standard Nyamagasani. All interspecific and important intraspecific inversions are shown.

Fig. 38 "Bandama" showing a dark staining heterozygous inclusion in IIIL arm.

Fig. 39 "Nile" sequence, III L-2 (fixed).

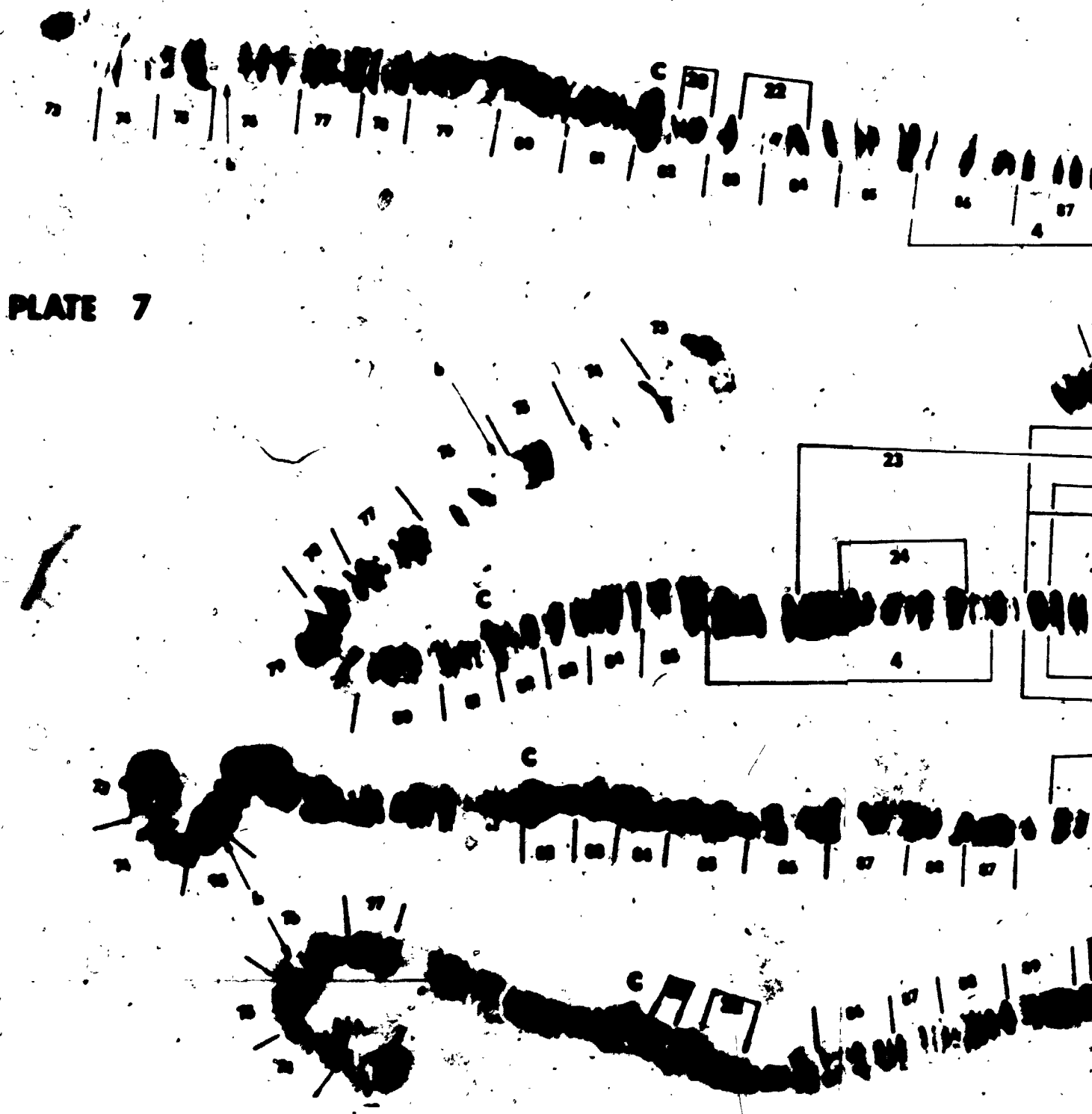
Fig. 40 "Bandama" sequence, III L-4 (fixed) and homozygous for the virtually fixed III L-2.17. "Soubre" and "Bandama" share both these arrangements. Limits of floating inversions of "Bandama" and "Soubre" are shown.

Fig. 41 "Nile" sequence showing III L-2 and the heterozygous III L-7. Floating III L-6 is also shown.

Fig. 42 "Sirba" sequence showing homozygosity for III L-2.6. Limits of other floating inversions are shown.

1 of

PLATE 7



2 of 2

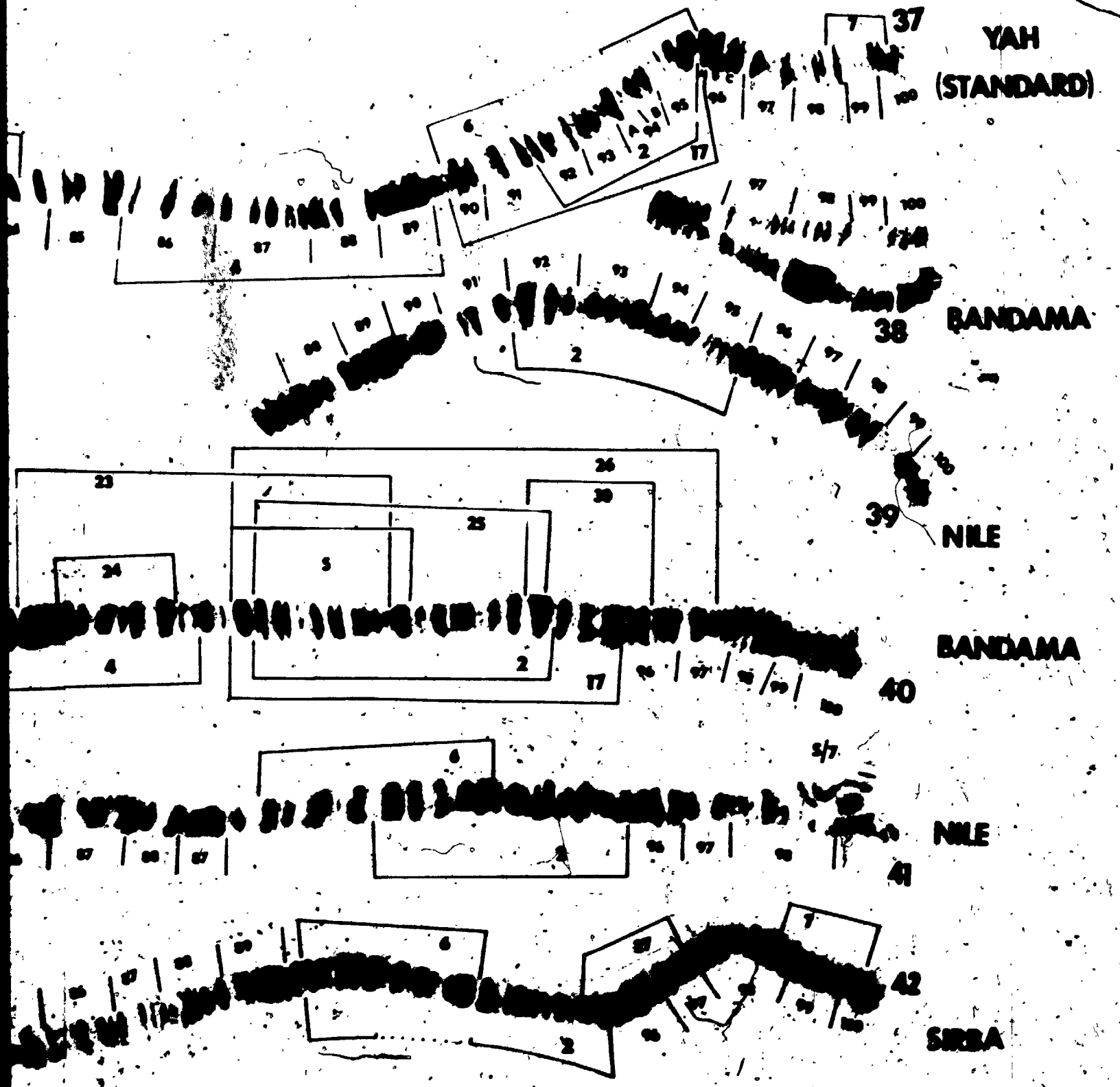

37
YAH
(STANDARD)
38
BANDAMA
39
NILE
40
BANDAMA
41
NILE
42
SIBBA

PLATE 8

"Yah" x "Bandama" F_1 hybrid.

Abbreviations as in Plate 1.

Fig. 43 Chromosome complement of F_1 hybrid.

Fig. 44 Interpretation of Fig. 43 showing interspecific and intraspecific inversions. Centric, ectopic pairing is shown in "Yah" constituents.

PLATE 8

PLATE 9

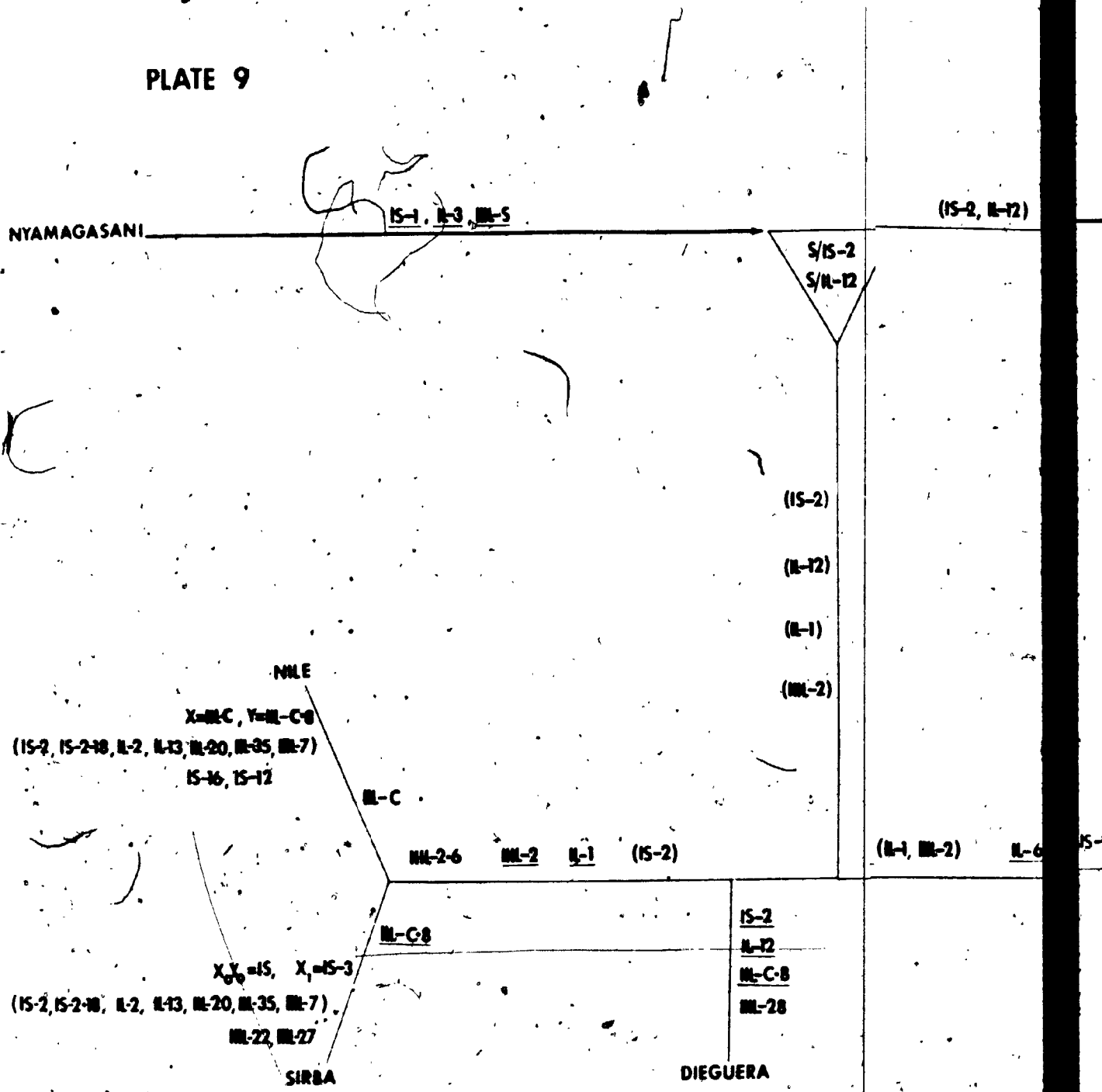
Phylogenetic relationships among the seven taxa included in the S. damnosum complex. Starting with "Nyamagasani" (arrow) an ancestral polymorphism is assumed for IS-2 and IL-12 (triangle).

Inversions shown along lines leading to lineage groups are of three kinds: inversions in brackets are shared among groups. Underlined inversions (interspecific) and non-underlined inversions (intra-specific) are common to the following species.

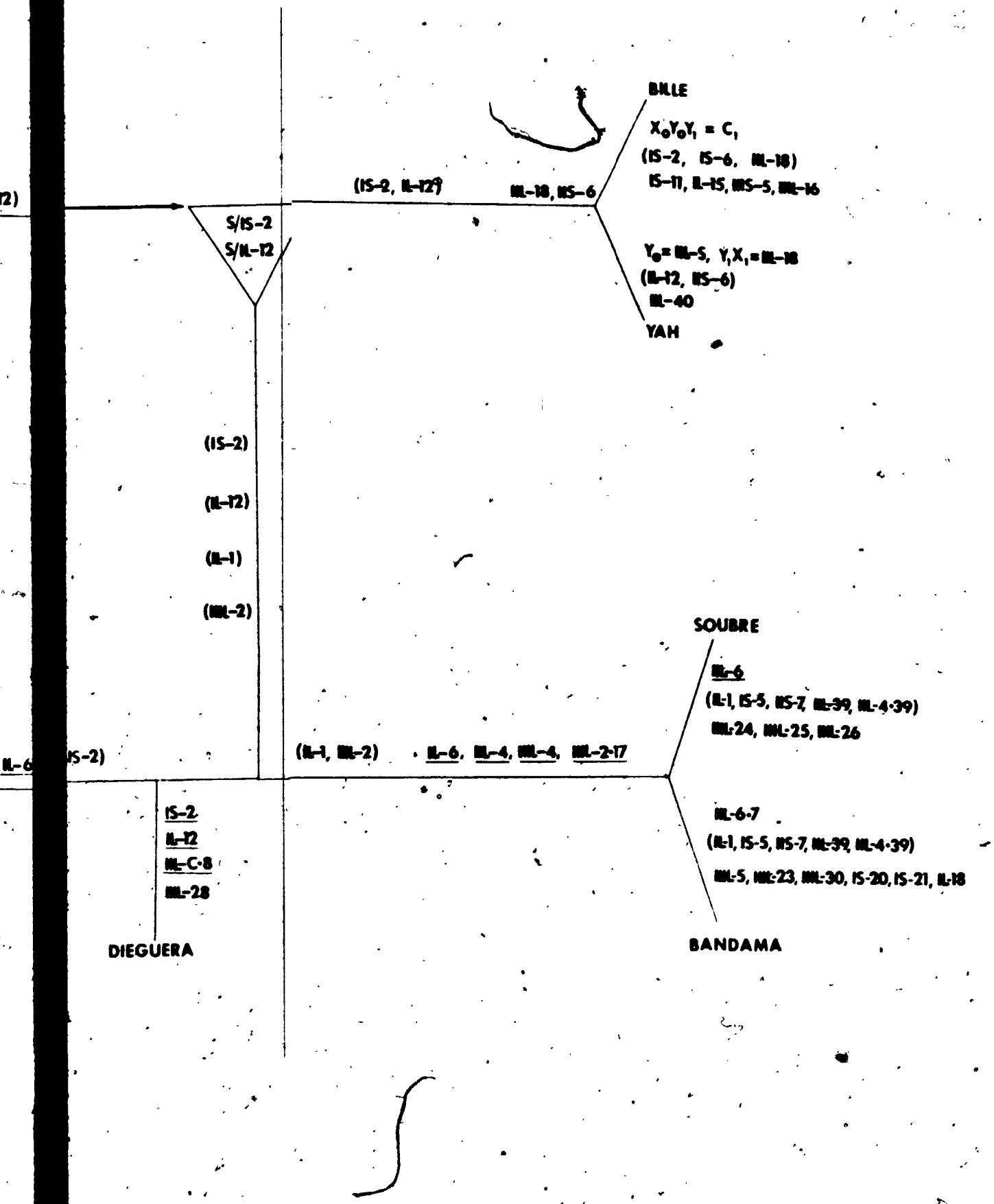
For each species sex chromosomes, shared inversions (bracketed) and endemic inversions are shown. The origin of the phylogeny i.e. the ancestral sequence is not determined.

1 of

PLATE 9


NYAMAGASANI
IS-1, IL-3, IML-5
(IS-2, IL-12)
S/IS-2
S/IL-12
(IS-2)
(IL-12)
(IL-1)
(IML-2)
NILE
X=IML-C, Y=IML-C-8
(IS-2, IS-2-18, IL-2, IL-13, IML-20, IML-35, IML-7)
IS-16, IS-12
IML-C
IML-2-6
IML-2
IL-1
(IS-2)
(IL-1, IML-2)
IS-2
IL-12
IML-C-8
IML-28
IML-C-8
X0Y0=IS, X1=IS-3
(IS-2, IS-2-18, IL-2, IL-13, IML-20, IML-35, IML-7)
IML-22, IML-27
SIRBA
DIEGUERA

2 of 2


BILLE
$X_0Y_0Y_1 = C_1$
(IS-2, IS-6, IIL-18)
IS-11, IL-15, IIS-5, IIL-16
(IS-2, IL-12)
IIL-18, IIS-6
S/IS-2
S/IL-12
Y_0= IIL-5, Y_1X_1= IIL-18
(IL-12, IIS-6)
IIL-40
YAH
(IS-2)
(IL-12)
(IL-1)
(IIL-2)
SOUBRE
IIL-6
(IL-1, IS-5, IIS-7, IIL-39, IIL-4-39)
IIL-24, IIL-25, IIL-26
(IL-1, IIL-2)
IL-6, IL-4, IIL-4, IIL-2-17
IS-2
IL-12
IIL-C-8
IIL-28
DIEGUERA
IIL-6-7
(IL-1, IS-5, IIS-7, IIL-39, IIL-4-39)
IIL-5, IIL-23, IIL-30, IS-20, IS-21, IL-18
BANDAMA